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Weeds

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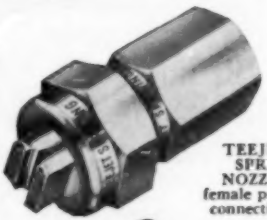

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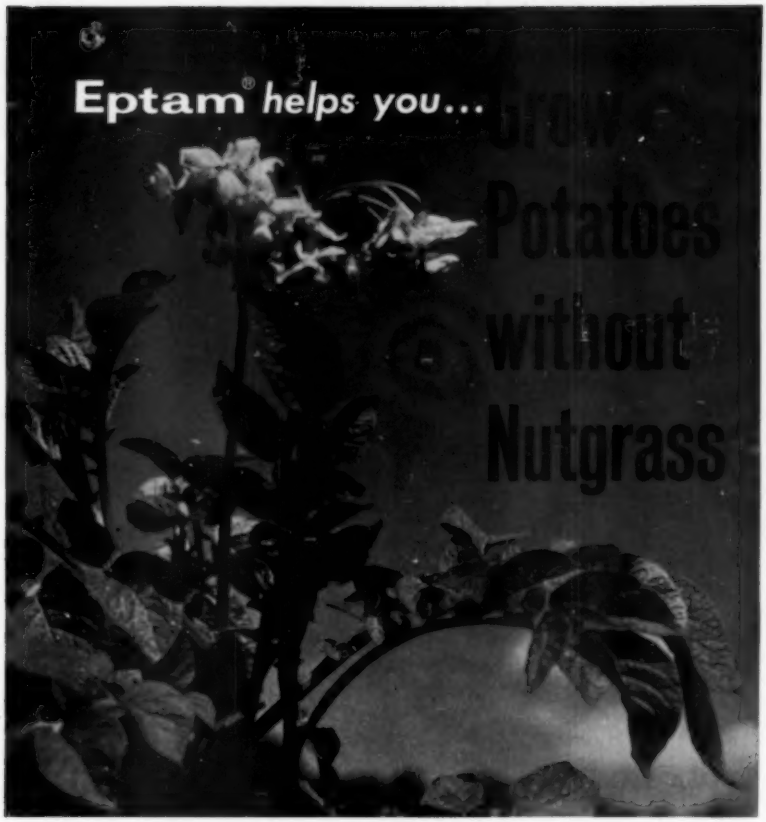

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Weeds

JOURNAL OF THE WEED SOCIETY OF AMERICA

VOLUME 9 APRIL 1961 NUMBER 2

Penetration of Living and Non-living Surfaces by 2,4–D as Influenced by Ionic Additives[1]

S. S. SZABO and K. P. BUCHHOLTZ[2]

THE herbicidal effects of 2,4–dichlorophenoxyacetic acid (2,4–D) have been shown to be influenced by a number of factors. Among these (2, 3) are: (a) intensity of light before and after treatment, (b) stage of development and growth of the plant or tissues at time of treatment, (c) reserve carbohydrate supply, (d) temperature, (e) available soil moisture, (f) pH of the solution, (g) surface active agents, and (h) formulation.

Considerable advances in the use and adaptability of 2,4–D have been made thus far. Further advances in the use and application of this and related compounds can be anticipated as additional knowledge is gained relative to the mechanism of herbicidal action, the nature of species and varietal selectivity, the specificity of reaction of diverse compounds, and the means of suppressing or intensifying particular biological effects. The processes whereby these compounds penetrate and move within the plant undoubtedly are of great significance. The interpretations of results have frequently been complicated by an inability to fully evaluate the action mechanisms.

The herbicidal effects of 2,4–D have been shown to be influenced by various ionic additives (4, 5). It has been demonstrated that the ammonium and phosphate ions will increase the herbicidal effects of 2,4–D, whereas the various metallic ions as iron, copper, and zinc will decrease the herbicidal effects of 2,4–D (1, 5). The purpose of this study was to ascertain the effect of ionic additives upon the penetration of 2,4–D in solution at various pH levels through artificial and living membranes. These results were compared with similar treatments applied directly to the leaf surfaces of growing plants.

[1]Received for publication Jan. 20, 1959. Contribution from the Dept. of Agronomy, Wisconsin Agr. Exp. Sta., Madison, Wisconsin. Published with the approval of the Director of the Wisconsin Agr. Exp. Sta. Part of a thesis submitted by the senior author to the Graduate School of the University of Wisconsin in partial fulfillment of the requirements for the Ph.D. degree.

[2]Formerly Research Assistant in Agronomy, University of Wisconsin now Assistant Professor of Agronomy, New Mexico State University, University Park, New Mexico and Professor of Agronomy, University of Wisconsin, Madison, Wisconsin, respectively.

MATERIALS AND METHODS

The ionic additives used were: NH_4^+ as NH_4NO_3, Co^{++} as $CoNO_3.6H_2O$, Cu^{++} as $CuSO_4.5H_2O$, Fe^{++} as $FeC_4H_4O_6$, Mn^{++} as $MnNO_3$, PO_4^{---} as Na_2HPO_4, and Zn^{++} as $ZnNO_3.6H_2O$. They were combined individually with solutions of the triethanolamine salt of 2,4–D. All solutions were adjusted to the desired pH with 0.1 N HCl and 0.1 N NaOH.

A series of observational experiments were performed in the field wherein the sulfate and nitrate forms of NH_4^+, Co^{++}, Cu^{++}, Fe^{++}, Mn^{++}, and Zn^{++} were compared. Comparable results were obtained with both the sulfate and nitrate forms of these ions. The $CuSO_4.5H_2O$ was used in these experiments since it was most readily available. The $FeC_4H_4O_6$ (ferrous tartrate) was used since the nitrate and sulfate salts of iron were unstable in solution and broke down rapidly. The Na_2HPO_4 was used because of its ease of solubility and stability in solution. Other phosphate salts tended to precipitate from solution. Although it was not used, H_3PO_4 would probably have been a good source of the PO_4^{---}ion.

Experiment 1. Figure 1 illustrates part of the apparatus used for the measurement of 2,4–D penetration through membranous surfaces. Four ml of each solution, containing 750 mg/l of the triethanolamine salt of 2,4–D plus sufficient ion-containing salt to provide 1500 mg/l of one of the ions, were placed in small screw-cap vials. The inside diameter of the open ends of these vials was 9 mm. Pieces of collodion membrane or the upper leaf epidermis of the Crassulacean plant, *Sedum*, were placed across the open ends of the vials and were secured tightly in place with screw caps from which the ends had been sanded off. The *Sedum* epidermis was obtained by peeling it from the sub-epidermal tissue. Each screw-cap vial was then inverted and supported in a 20 ml shell vial containing 10 ml of double-distilled water. Each screw-cap vial was supported within the shell vial by means of a flat piece of flexible rubber containing a hole slightly smaller in diameter than the screw-cap vial itself. The rubber support permitted easy adjustment of the screw-cap vial and also formed an air-seal across the top of the shell vial, preventing evaporation. The screw-cap vials were supported within the shell vials so that the membrane-covered ends were immersed in the water to a depth of approximately 2 mm. The screw-cap vials, containing the 2,4–D plus ionic additive, were left in contact with the water in the shell vials for 4 hours. The screw-cap vials were then removed and the shell vials were stoppered to prevent evaporation. The concentration of 2,4–D in the shell vials was determined shortly thereafter with a Beckman spectrophotometer at a wave length of 2835 Angstroms. All phases of this experiment were conducted at approximately 25°C and under normal artificial lighting conditions.

Experiment 2. The various ionic additives were combined with 2,4–D and applied to the cotyledonary leaves of sunflower, *(Helianthus annuus)* variety Mammoth Russian, and snap beans, *(Phaeseolus*

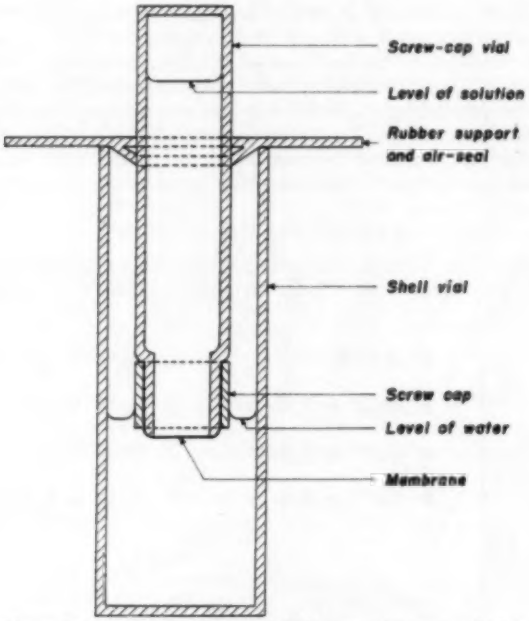

Figure 1. Sectional diagram showing arrangement of the vials used for conducting penetration studies.

vulgaris) variety Tendergreen. Each solution was distributed evenly over the upper surface to the leaf in 10 drops of 0.01 ml each, resulting in an application of 50 micrograms of additive and 125 micrograms of 2,4–D per leaf. Higher concentrations of either the additives or the 2,4–D caused injury to the absorbing surfaces of the leaves. Thus, the solution concentrations used in the second experiment were lower than the solution concentrations used in the first. Four hours after treatment, each treated leaf was washed five times with a 5 ml portion of double-distilled water. The wash water had been previously adjusted to pH 9 in order to facilitate removal of all remaining 2,4–D from the leaf surface. The concentration of 2,4–D in the various wash solutions was again determined spectrophotometrically. The apparent penetration of 2,4–D was then determined by the difference between the amount of 2,4–D originally applied and that recovered in the wash water.

Experiment 3. The absorption spectra of the various additives in combination with 2,4–D were analyzed by means of a Cary recording spectrophotometer. This was done in order to determine if 2,4–D reacted with any of the various additives to form chelate structures or other complexes that might impair the assay of 2,4–D. In this study, 50 mg/l of 2,4–D were combined with 20 mg/l of the individual ionic additives.

Experiment 4. Previously, it had been demonstrated by Dorschner[3] that the toxicities of various growth regulators may be correlated with the wettability characteristics of the compounds on various wax surfaces. Therefore, the surface tension of each of the solutions used in the preceding experiments was determined by means of a Model 70520 Cenco du Nouy tensiometer. Contact angles for these solutions were measured by means of a Model 3022 Bausch and Lomb microprojector. The method used was as described by Dorschner.

RESULTS AND CONCLUSIONS

Experiment 1. Figure 2 illustrates the effect of ionic additives upon the penetration of 2,4–D through a collodion membrane at

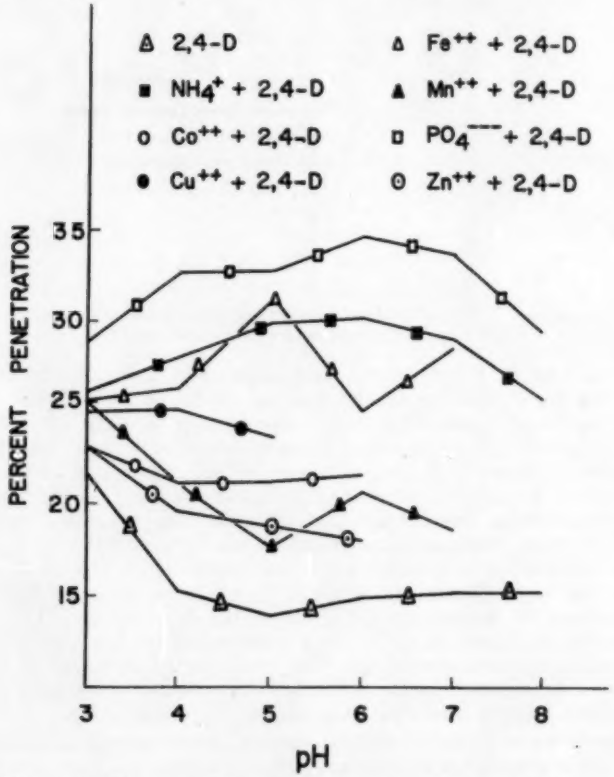

Figure 2. The penetration of collodion membrane by 2,4–D as influenced by pH and various ionic additives.

[3]Dorschner, K. P. Some factors influencing the herbicidal activity of plant growth regulating chemicals applied to alfalfa seedlings. Ph.D. thesis, University of Wisconsin, 1954.

various pH levels. Penetration of the 2,4–D alone was greatest at pH 3 and the least at pH 5, 21.0 and 14.0 percent, respectively. A small increase in penetration was observed between pH 5 and pH 8.

All of the additives increased the penetration of 2,4–D through the collodion membrane at all pH levels observed as compared to 2,4–D alone. The solutions containing the Co^{++}, Cu^{++}, Mn^{++}, and Zn^{++} additives showed the greatest penetration of 2,4–D at pH 3. The Fe^{++} additive gave an erratic penetration curve, showing the greatest penetration of 2,4–D at pH 5. The reason for this erratic behavior is unknown. The NH_4^+ and PO_4^{---} additives greatly enhanced the 2,4–D penetration, giving inverted parabolic curves with the greatest amount of penetration at pH 6 in both instances. Penetration values were not obtainable for all pH values with some of the metallic ionic additives, since they precipitated from solution at pH levels above pH 6. The Cu^{++} additive precipitated from solution above pH 5.

Table 1 shows the percent penetration through *Sedum* epidermis of 2,4–D in solutions of 750 mg/l of 2,4–D in combination with 1500 mg/l of the various ionic additives at two pH levels. At pH 3, NH_4^+ and PO_4^{---} increased the penetration of 2,4–D, whereas Fe^{++} caused a marked reduction when compared to 2,4–D alone. The other ionic additives were without effect. The penetration of 2,4–D

Table 1. Percent penetration through *Sedum* epidermis, during a 4 hour period, by solutions of 750 mg/l of 2,4–D in combination with ionic additives at two pH levels.

Ionic additive 1500 mg/l	Per cent penetration	
	pH 3	pH 5
None	16.1	9.3
NH_4^+	23.5	13.5
Co^{++}	16.5	9.8
Cu^{++}	14.7	0.0
Fe^{++}	3.0	0.0
Mn^{++}	16.0	0.0
PO_4^{---}	21.1	12.7
Zn^{++}	17.6	8.3

at pH 5 was decreased for all solutions when compared to the penetration at pH 3. Compared to the penetration of 2,4–D alone, NH_4^+ and PO_4^{---} increased the penetration of 2,4–D, whereas Fe^{++} and Cu^{++} completely inhibited the penetration of 2,4–D. Co^{++}, Zn^{++}, and Mn^{++} did not alter the penetration of 2,4–D in this experiment.

Experiment 2. The apparent penetration through bean and sunflower cotyledonary leaf surfaces by solutions of 2,4–D in combination with various ionic additives, is presented in Table 2. With the snap beans at pH 3 only the Fe^{++} and Cu^{++} additive solutions gave 2,4–D penetration values which differed markedly from the penetration of the 2,4–D solution alone. The Cu^{++} caused an increase whereas the Fe^{++} additive caused a decrease in the penetration of 2,4–D. At pH 5, all of the ionic additives except NH_4^+ and PO_4^{---}

Table 2. Apparent percent penetration, during a 4 hour period, of bean and sunflower cotyledonary leaf surfaces by 125 micrograms of 2,4–D in combination with ionic additives at two pH levels.

Ionic additive 50 micrograms	Per cent penetration			
	Bean		Sunflower	
	pH 3	pH 5	pH 3	pH 5
None	49.6	46.0	42.0	14.9
NH_4^+	48.0	76.0	41.6	55.6
Co^{++}	50.0	10.0	77.2	26.4
Cu^{++}	66.0	4.0	78.0	0.0
Fe^{++}	38.0	30.0	48.0	0.0
Mn^{++}	50.0	4.0	49.6	24.0
PO_4^{---}	50.8	77.2	77.6	40.0
Zn^{++}	47.2	6.0	44.5	25.7

decreased the penetration of 2,4–D over corresponding values at pH 3. NH_4^+ and PO_4^{---} increased the penetration of 2,4–D.

Only the Co^{++}, PO_4^{---}, and Cu^{++} affected the 2,4–D penetration of sunflower extensively, at pH 3. These ionic additives increased the penetration of 2,4–D considerably. At pH 5, Cu^{++} and Fe^{++} completely inhibited the penetration of 2,4–D, whereas NH_4^+ and PO_4^{---} gave considerable increases. Mn^{++}, Zn^{++}, and Co^{++} appeared to increase the penetration of 2,4–D compared to 2,4–D alone; however, these penetration values were less than the corresponding values at pH 3.

Experiment 3. The results of the absorption spectra studies showed that only the solution containing Fe^{++} and 2,4–D gave indication of complex formation. Complex formation with Fe^{++} was indicated by a reduction in the height of the peak of the 2,4–D absorption spectrum curve, as illustrated in Figure 3, which amounted to an apparent reduction in the 2,4–D concentration of 13 percent. However, this masking of the 2,4–D concentration was insufficient to account for the observed inhibition of 2,4–D penetration by this additive.

Experiment 4. Some small differences in wettability properties were noted for the various solutions used as indicated in Table 3. However, no correlation was found between the wettability properties of any particular solution and the penetration results observed.

Table 3. Comparison of the surface tension, contact angle, and spreading coefficient of a 2,4–D solution and solutions of 2,4–D plus various ionic additives.

lb/A of 2,4–D in 40 gpa soln	Additive	lb/A of ionic additive in 40 gpa soln	Surface tension dynes/cm	Contact angle, degrees	Spreading coefficient
0.50	None	0.00	98.0	53.4	−39.5
0.50	NH_4^+	1.00	96.2	52.7	−37.8
0.50	Co^{++}	1.00	95.5	54.8	−40.4
0.50	Cu^{++}	0.25	119.2	57.5	−55.2
0.50	Fe^{++}	0.50	98.6	52.7	−38.8
0.50	Mn^{++}	1.00	98.0	53.5	−39.8
0.50	PO_4^{---}	1.00	97.1	58.1	−46.0
0.50	Zn^{++}	0.25	100.2	56.9	−45.4

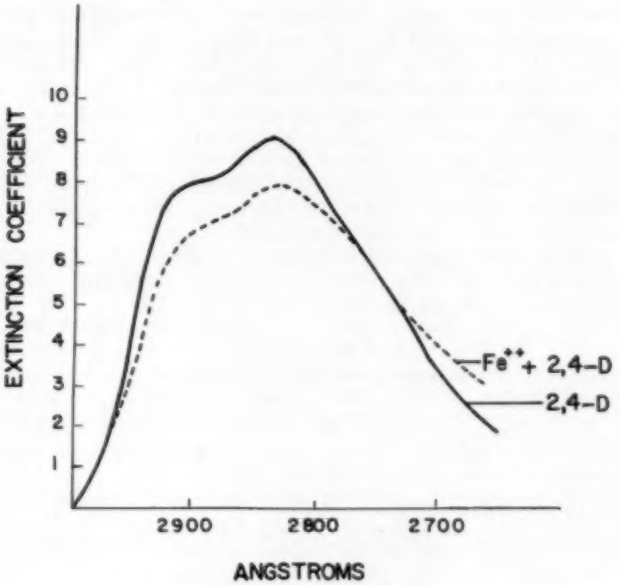

Figure 3. The absorption spectra of solutions of 2,4–D and 2,4–D in combination with iron.

SUMMARY

1. Studies were conducted to determine the effects of ionic additives upon the penetration of 2,4–D through a collodion membrane and *Sedum* epidermis. These results were compared to penetration studies conducted with living plants. Analyses were made of the absorption spectra and wettability properties of the various solutions containing 2,4–D and ionic additives.

2. All of the ionic additives increased the penetration of 2,4–D through the collodion membrane. The metal ions increased the penetration of 2,4–D to a greater extent at low pH than at high pH. The NH_4^+ and PO_4^{---} additives yielded parabolic-type penetration curves with increasing pH, with the greatest penetration at pH 6.

3. The studies with *Sedum* epidermis indicated an increased penetration of 2,4–D with the NH_4^+ and PO_4^{---} additives at both pH 3 and pH 5. The Fe^{++} and Cu^{++} additives caused a decreased penetration of 2,4–D at both pH values. The other ionic additives were without effect. The penetration was always less at pH 5 than at pH 3.

4. The results of the studies with bean and sunflower indicated relatively little effect of the ionic additives upon the penetration of 2,4–D at pH 3. The Fe^{++} and Cu^{++} additives were exceptions in

the bean tests, resulting in decreased and increased 2,4–D penetration, respectively. At pH 5, NH_4^+ and PO_4^{---} increased the penetration of 2,4–D into both bean and sunflower plants considerably. All of the metal ions decreased the penetration of 2,4–D into bean plants, whereas only Cu^{++} and Fe^{++} resulted in reduced 2,4–D penetration into the sunflower plants at this pH.

5. Analysis of the absorption spectra of the various solutions indicated that only the solution containing Fe^{++} and 2,4–D gave indications of complex formation. This apparent complex formation was believed to be inconsequential.

6. Analysis of the wettability properties of the various solutions indicated a lack of correlation between the wettability properties and the penetration effects observed.

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Some Factors Affecting the Control of Wild Oats with Barban[1]

H. A. Friesen[2]

"Selective" herbicidal properties of 4–chloro–2–butynyl *N*–(3–chlorophenyl)carbamate, barban,[3] when applied postemergence for the control of wild oats in cereal grains, have been observed in preliminary trials by a number of workers (1, 2, 3, 4, 5, 6, 7, 9, 10). Their results suggested that the degree of control was a function of several factors including dosage, volume of diluent, fertility of the soil and the stage of growth of both the weed and crop plants at the time of spraying. Differences in tolerance between grain crops and between varieties within a crop were also indicated.

The control of wild oats with barban is complicated by the wide range of conditions under which this weed thrives, its irregular habit of germination and the seemingly critical limits of herbicidal tolerance of both weed and crop under these varying conditions. To investigate the interdependence of these factors on the control of wild oats with barban a series of experiments were conducted at the Lacombe Experimental Farm in 1959 and 1960. The reaction of the main seeded crop, wheat, and the differential response of three varieties of wheat, oats, barley and flax were also studied. The work was conducted both in the greenhouse and the field, first to confirm the theoretical response under controlled conditions and second to measure control under actual field conditions.

Materials and Methods

Greenhouse experiments. During the winter 1958–59, wild oats and Olli barley were planted separately in 4-in. waxed cartons, thinned to three plants per carton and sprayed with barban at 0, 4, 8, and 16 oz/A using water as the diluent at 6.5 and 56.0 gal/A. Both the weed and crop were in the 1½-leaf stage of growth when sprayed. Plant heights and weights were taken at heading time of the barley. A random arrangement with four replications was used. In 1959–60 a series of trials was conducted to study the effect of spray pattern, as well as volume, with barban applied at different dosages to wild oats and barley at three stages of growth. The weed and crop were planted in the same pots. Mixtures of barban with various formulations of 2,4–D and MCPA were also included in this study. The spray patterns and volumes were obtained by the use of different types of nozzles and a variable speed turntable. The nozzles used are referred to by their Spraying Systems Co. numbers in Table 2. The double fan was achieved by spraying the plants twice, first with

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the nozzle tilted 45° forward and again when tilted 45° backward. An operating pressure of 30 psi was used in all tests. Height measurements were taken on all plants at 4-day intervals for a 6-weeks period beginning on the date of spraying.

Field experiments. In 1959 an experiment was conducted in the field to study the effect of dosage, volume of diluent and growth stage on the control of wild oats in wheat with barban. The plot design was a triple lattice having 16 treatments and three replicates. The treatments consisted of four dosage levels (0, 4, 8, and 16 oz/A) at two volumes of diluent (6 and 30 gal/A) applied at the two growth stages (1–1½ and 2½–3-leaf) of the wild oats. A plot sprayer equipped with Spraying Systems Nos. 730077 and 730462 fan type nozzles to apply the 6 and 30 gal/A of solution, respectively, was used. The 48 plots, 8 x 12 feet in size, were set out in an area very heavily infested with wild oats and seeded to Thatcher wheat. The treatments were assessed by: (1) five wild oats plants in each plot measured for height at five intervals during the growing season; (2) plant counts and dry weights of the wild oats taken on 2 square yards per plot at maturity of the wheat; (3) the yield of wheat; and (4) germination tests of the wild oats seeds.

In 1960, barban was field tested at the same rates used in 1959. However, three growth stages, viz: 1½–2, 2½–3 and 3½–4-leaf were used and the herbicide was applied with two types of nozzles, Spraying Systems hollow cone X–4 and fan No. 650067. Each type of nozzle delivered 5 gal/A of spray solution when operated at a pressure of 45 psi and a speed of 4 mph. A split plot design with three replicates was used. Assessment was the same as in 1959. The wild oats infestation was considerably lighter and less uniform than in 1959.

Fertility level. A 2-acre field heavily infested with wild oats was seeded to Thatcher wheat in 1959. One-half of the field was fertilized with 50 lb/A of ammonium phosphate, 11–48–0. When the majority of the wild oats had 1½ leaves, the field, with the exception of a check strip, was sprayed with barban at 8 oz/A mixed with triethanolamine salt of MCPA at 4 oz/A in 5 gal/A of water. Assessment was made by plant counts of both the weed and crop, dry weight of wild oats and yield of wheat taken in 12 one-square-yard samples on each strip. A similar trial was conducted in 1960 except that barban alone at 4 oz/A was used.

Varietal tolerance. Three varieties of wheat, barley, oats, and flax were sprayed with barban at 8 and 32 oz/A in 6 gal/A of water at the 2- and 5-leaf stages in 1959. Quadruple rod row plots were used in a randomized block design with four replicates. The very heavy rate of barban was used to ensure injury to the crops since their tolerance limits were not known. Because only crop reaction was to be studied, the plots were kept handweeded throughout the season. Assessment was made by yield of grain at maturity. As the results of this test suggested the need for a more detailed study of the reaction of Thatcher and Selkirk wheat, a study was made in 1960 of these two varieties following treatment with 0, 4, 8, and 16 oz/A

of barban applied with cone and fan type nozzles at three different growth stages. A split plot design with four replicates was used. The plot area was free of wild oats in both years.

RESULTS AND DISCUSSION

Greenhouse experiments. All barban treatments in the greenhouse suppressed the growth of both the wild oats and the barley (Table 1). At the heading stage of the barley, when all plants were cut, dried and weighed, new leaf and stem initials were in evidence on

Table 1. Weight of wild oats and Olli barley treated with barban in the greenhouse, 1958–59.

Barban oz/A	Volume gal/A	Barley lb/A	Wild oats lb/A
4	6.5	896	96
8	6.5	432	64
16	6.5	96	16
0		1536	416
4	56	1424	96
8	56	992	80
16	56	608	32

the wild oats plants sprayed at 4 oz/A and on 50 per cent of those sprayed at 8 oz/A in 6.5 gal/A of water. At this time, none of the wild oats treated with 16 oz/A in 6.5 gal/A of water were dead, but it was significant that none had initiated further growth. Under greenhouse conditions the two lighter dosages reduced the growth of barley severely but only at 16 oz/A was normal heading prevented. Increasing the volume of diluent to 56 gal/A reduced the reduction of growth effect of the two heavier dosages on wild oats and greatly reduced this effect on the barley at all dosages used. There was no obvious reason for the lack of differential suppression of the wild oats due to the volumes of diluent used with 4 oz/A.

In both the greenhouse and field experiments, in 1959, spray volume was increased by the use of larger nozzles, which resulted in larger spray droplets. This suggested that spray pattern rather than actual volume might be responsible for the observed inverse ratio of effectiveness to volume of diluent. In 1960, spray patterns from the cone and double fan nozzles clearly increased the effectiveness of the 2 and 4 oz/A dosages (Table 2). With the 8 oz/A dosage this advantage was lost. Barley showed no significant decrease in height or weight as the result of the dosages or the nozzles used to apply them, with but one exception, 8 oz/A applied with the cone nozzle.

Plant height measurements showed a near cessation of growth of the treated wild oats for at least four weeks following treatment. Recovery of surviving plants was most rapid and complete at the lower dosages and higher spray volumes. This agrees generally with the field results and the findings of Hoffman et al (8).

The mixture of barban and the butyl ester of 2,4–D, each at 4 oz/A, tended to decrease the control of wild oats observed from the

Table 2. Effect of spray patterns and volumes on barban at different rates and barban plus 2,4–D for wild oats control, winter 1959–60.

Barban oz/A	Butyl ester 2,4–D oz/A	Cone Y-2 Spray volume 6 gal/A		Fan 730039 Spray volume 5 gal/A		Fan 730077 Spray volume 10 gal/A		Double fan 730039 Spray volume 10 gal/A	
		Ht in	Wt lb/A	Ht in	Wt lb/A	Ht in	Wt lb/A	Ht in	Wt lb/A
2	0	10.0	122	12.4	188	12.7	280	7.6	160
4	0	5.4	83	6.6	117	11.2	150	5.4	95
8	0	5.3	62	4.6	42	4.9	40	4.7	65
4	4	6.0	82	8.0	162	8.6	190	4.9	85
Untreated								24.6	520

L.S.D. at 5% and 1% for weight of wild oats 11 and 14 lb/A, respectively.

use of a comparable dosage of barban alone (Table 2). Similar results were obtained from mixtures with the triethanolamine salt of MCPA and the sodium salt of 4–(MCPB).

The 1960 greenhouse data in Table 3 confirm the 1959 field results in that the best control of wild oats was realized from the early spraying. The differences in wild oats weights from types of nozzles was not significant.

Table 3. Effect of barban at 4 oz/A on wild oats and barley in the greenhouse, winter 1959–60.

Growth stage	Nozzle type	Wild oats		Barley	
		Ht in	Wt lb/A	Ht in	Bu/A
1-leaf	Fan 730077	9.3	210*	33.1	46.6*
3-leaf	Fan 730077	18.7	460*	27.5	36.0
4-leaf	Fan 730077	25.7	490	26.2	30.8
Untreated		24.6	520	28.3	34.8
1-leaf	Cone Y-2	4.5	190*	30.5	44.0*
3-leaf	Cone Y-2	18.1	430*	28.4	33.6
4-leaf	Cone Y-2	26.9	540	26.4	27.4
L.S.D. 5%			34		2.8

Field experiments.

Effect on wild oats. All barban treatments resulted in a near cessation of growth of the wild oats for about 4 weeks following spraying. The leaves of the treated plants became thickened and assumed a deep blue color. Later the leaves became necrotic and some of the plants died. The extent of this injury or in other words the degree of control was dependent upon dosage, growth stage at spraying time and spray volume.

In both 1959 and 1960, the greatest reduction of growth, expressed as dry weight, and the greatest reduction in numbers of plants resulted from the two higher dosages applied at the early, 1½–2 leaf, stage of growth (Tables 4 and 5). Suppression of growth was manifest in fewer tillers per plant and reduced height and development of these tillers. These surviving plants resumed active growth usually by the initiation of new tillers about 4 weeks after

Table 4. Effects of barban on wild oats and wheat, Lacombe, Alberta. 1959.

Growth stage wild oats	Rate oz/A	Volume gal/A	Wild oats			Wheat	
			Plants per sq yd	Dry wt lb/A	Germination pct	Plants per sq yd	Bu/A
Untreated	None	None	351	242	75	116	22.8
1–1½-leaf	4	6	360	219	50	153	29.9
1–1½-leaf	8	6	280	137	44	130	26.8
1–1½-leaf	16	6	236	125	63	102	31.1
1–1½-leaf	4	30	338	203	81	111	20.2
1–1½-leaf	8	30	332	237	79	129	20.9
1–1½-leaf	16	30	270	179	66	118	21.8
2½–3-leaf	4	6	338	201	46	125	26.4
2½–3-leaf	8	6	314	150	36	128	25.8
2½–3-leaf	16	6	286	113	58	111	22.2
2½–3-leaf	4	30	294	231	73	121	21.1
2½–3-leaf	8	30	367	246	74	106	25.9
2½–3-leaf	16	30	336	298	61	110	27.6
L.S.D. 5%				66			6.3
1%				89			9.3

spraying. Plant height measurements begun at this time in 1959 and continued for a 6-weeks period showed that the recovery and growth of the surviving plants decreased with increasing dosage, spray volume and stage of growth (Figure 1). The reduction in number and dry weights of plants also decreased with increasing plant age in both seasons. However, in 1960, the reductions, particularly in numbers of plants, were greater than in 1959 (about 10 per cent), when similar treatments were compared. Inability to differentiate accurately between wheat and wild oats seedlings at spraying time precluded the obtaining of data on the numbers of plants actually killed. While these results support the findings of Hoffman et al. (8), the significant reduction in dry weight from 16 oz/A of barban at the 3½–4 leaf stage indicated a longer susceptible period than that suggested by those workers.

Table 5. Effect of barban on wild oats and wheat, Lacombe, 1960.

Growth stage wild oats	Barban oz/A	Wild oats		Wheat	
		Plants sq yd	Dry wt lb/A	Plants sq yd	Yield bu/A
Untreated		40	207	123	29.7
1½–2-leaf	4	21	136*	131	31.2
1½–2-leaf	8	13	96*	126	33.9*
1½–2-leaf	16	12	64*	119	29.2
2½–3-leaf	4	42	234	127	33.0*
2½–3-leaf	8	26	146*	127	35.0*
2½–3-leaf	16	13	72*	130	34.8*
3½–4-leaf	4	36	223	118	28.6
3½–4-leaf	8	27	177*	120	31.6
3½–4-leaf	16	20	93*	109	32.4*
LSD 5%, rates			21		2.3

LSD 5% for stage and rate x stage interaction were not significant.

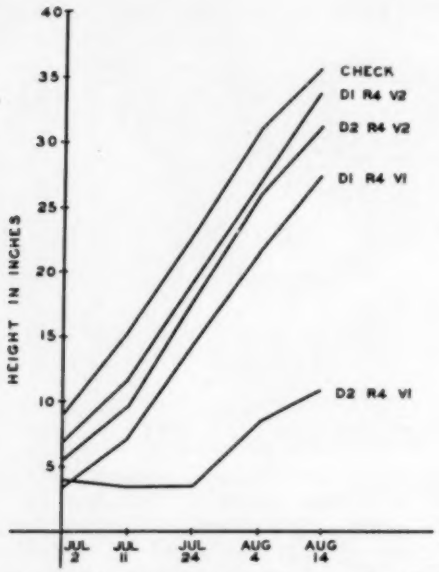

Figure 1. Growth rate of wild oats as influenced by treatments with barban applied June 8 (D_1–1½ leaf stage); and June 16 (D_2–2½–3 leaf stage); R_4–barban at 16 oz/A; V_1–6 gal/A of water; V_2–30 gal/A of water.

In 1959, increasing the volume of diluent from 6 to 30 gal/A significantly reduced the effectiveness of this herbicide (Table 4). The assumption that this behavior was due to droplet size and spray pattern rather than actual spray volume was not borne out in the 1960 field results, where the results of sprayings made with either the fan or hollow cone types of nozzles were not significantly different. Since the greenhouse data (Table 2) showed such a difference it is assumed that physiological or other factors masked this effect in the field.

Effects on wheat. Significant increases in the yield of wheat were realized in 1959 only where treatment was made in the low volume of diluent at the early growth stage. The control of wild oats, following 8 oz/A was nearly equal to that from 16 oz/A, each in 6 gal/A of water and sprayed at the 1–1½-leaf stage. The 16 oz/A at this stage caused visible injury to the wheat (delayed maturity and stunting) but this damage was more than offset by the control of wild oats. The 8 oz/A rate did not give a significant increase in the yield of wheat because of mechanical injury on one of the plots. The significant yield increase where 4 oz/A was used is not readily explained. However, all treatments resulted in a near cessation of growth for some 4 weeks during the early growth stages and this

coupled with a heavier crop stand on these plots possibly accounts for this increase. At the later growth stage, 4 and 8 oz/A in the low volume of diluent gave small but non-significant increases in the yield of wheat. The 16 oz/A dosage at this stage, while greatly suppressing the wild oats, also suppressed tillering of the wheat with the result that no increase in grain yield was obtained. This treatment also delayed the maturity of the wheat but early frosts prevented obtaining data in actual days.

At the early growth stage where the high volume of diluent was used none of the treatments increased the yield of wheat. At the later, 2½–3 leaf stage the two heavier dosages of barban in the high volume of water resulted in small increases in the yield of wheat.

Because of the very light infestation of wild oats, especially on two of the replicates, increases in the yield of wheat as the result of their control were not large in 1960. Barban applied at 8 oz/A at the 1½–2-leaf and at 4, 8 and 16 oz/A at the 2½–3-leaf stages of the wild oats significantly increased the yield of wheat. The 16 oz/A dosage caused visible injury at all stages but was most severe at the earliest stage.

This discrepancy between the 1959 and 1960 wheat yields at the early 1½–2-leaf stage may be attributed to the very much lighter infestation of wild oats in 1960 and to the relative difference in stage of growth of weed and crop. In 1959 the wild oats and wheat were at the same growth stage when sprayed while in 1960 the wheat already had 3 leaves when the wild oats only had 1½–2 leaves. Of particular interest was the significant increase in the yield of wheat due to spraying with barban at 16 oz/A at the 3½–4 leaf stage in 1960.

As shown in Table 4, none of the treatments had a measurable effect on the germination of the wild oats seeds which matured.

Fertility effects. In 1959 there was a greater reduction in the number of plants and dry weight of wild oats on the fertilized wheat treated with the mixture of barban and MCPA than where this mixture was sprayed on wheat seeded without the fertilizer (Table 6). There also was a substantial increase in the yield of the fertilized wheat due to

Table 6. Fertility of the soil effect on the control of wild oats in wheat with barban. 1959–60.

Year treated	Barban oz/A	MCPA oz/A	11–48–0 lb/A	Wild oats		Wheat bu/A
				Plants/ sq yd	Wt lb/A	
1959	8	4	50	80	43	38.5
1959	nil	nil	50	112	200	30.2
1959	8	4	nil	95	96	29.8
1960	4	nil	50	55	140	27.7
1960	nil	nil	50	635	1350	10.0
1960	nil	nil	nil	403	1130	11.9
1960	4	nil	nil	160	350	24.7

LSD at 5% was highly significant for all barban vs no barban comparisons for plants/sq yd, weight of wild oats and yield of wheat.

spraying. In 1960, under a much heavier infestation, the results from barban alone at 4 oz/A were similar but much more pronounced than in 1959.

Crop Varieties. In order to offset the inherent differences in yield potential between the different varieties within a crop the yields of three varieties of each of three crops treated with barban at two growth stages are expressed as a percentage of their respective

Table 7. Response of different crop varieties treated with barban at two growth stages. Lacombe, 1959.

Treatment		Barley		Wheat		Flax		
Rate oz	Stage of growth	Variety	Yield as % of check[1]	Variety	Yield as % of check[1]	Stage of growth	Variety	Yield as % of check[1]
Check		Gateway	78.4[1]	Thatcher	47.8[1]		Redwing	16.8[1]
8	2 leaf	Gateway	94.2	Thatcher	98.3	5 leaf	Redwing	118.0
8	5 leaf	Gateway	98.2	Thatcher	74.2	9 leaf	Redwing	95.4
Check		Husky	103.3	Saunders	47.0		Redwood	12.4
8	2 leaf	Husky	97.4	Saunders	103.8	5 leaf	Redwood	71.0
8	5 leaf	Husky	85.0	Saunders	81.9	9 leaf	Redwood	50.7
Check		Olli	69.4	Selkirk	45.4		Rocket	15.4
8	2 leaf	Olli	105.9	Selkirk	90.3	5 leaf	Rocket	92.0
8	5 leaf	Olli	71.6	Selkirk	92.4	9 leaf	Rocket	62.5

[1]Check (untreated) given as actual yield of grain in bushels per acre while the treatment yields are expressed as a percentage of their respective check yields.

untreated check yields (Table 7). All varieties of oats were so severely injured that the yields were not taken. Barley, wheat and flax appeared more tolerant to barban applied at the early growth stage, although the heavier rate at this stage usually reduced the yield. There were no differences between barley varieties sprayed at the 2-leaf stage at 8 oz/A but at 32 oz/A Olli appeared more susceptible. At the 5-leaf stage 8 oz./A greatly reduced the yield of the variety Olli.

In the wheats, barban at both dosages sprayed at the 2-leaf stage slightly reduced the yield of Selkirk. At the 5-leaf stage, Selkirk tended to show somewhat more tolerance to 8 oz/A of barban than did Saunders or Thatcher. There is no apparent explanation for this change in relative tolerance with advancing age of the plants.

In the 1960 experiment the yields of both Thatcher and Selkirk were significantly reduced by barban at 16 oz/A at the 1½ and 2½ leaf stages. As in 1959, at the 4-leaf stage only Thatcher was reduced in yield. Barban at 8 oz/A at the 1½-leaf stage significantly reduced the yield of both varieties but the yield of Selkirk was about 10 per cent lower than that of Thatcher.

The flax varieties appeared to be more susceptible than either barley or wheat except the variety Redwing. Redwood was the most susceptible flax variety.

SUMMARY

1. The effect of dosage, stage of growth, spray volume, spray patterns, and fertility of the soil on the selective control of wild oats in wheat with 4–chloro–2–butynyl *N*–(3–chlorophenyl)carbamate, barban, was studied under greenhouse and field conditions. The tolerance to this herbicide of three varieties each of wheat, barley, oats, and flax was also investigated.

2. Generally wild oats control decreased with increasing plant age. Reduction in the number of wild oats plants in the field was greatest, approaching 40 per cent in 1959 and 50 per cent in 1960, when barban was sprayed at 8 and 16 oz/A in a low volume of diluent. 6 gal/A of water, and the wild oats were in the 1½–2 leaf stage of growth. The development of the surviving plants was delayed and tillering greatly suppressed, with the dry weight of the treated wild oats significantly below that of the untreated plants. With the exception of barban at 16 oz/A in 1960, these treatments resulted in significant increases in the yield of wheat in both years.

3. These treatments applied to wild oats at the 3-leaf stage again reduced growth and tillering but fewer plants were killed. The yield of wheat treated with 8 oz/A of barban at this later stage was increased but was decreased by the 16 ounce rate in 1959. In 1960 both rates increased the yield of wheat.

4. In 1960, barban at 16 oz/A resulted in a significant reduction in wild oats weight even when spraying was withheld until the 3½–4 leaf stage.

5. Increasing the volume of diluent to 30 gpa water greatly reduced the effectiveness of barban applied at both growth stages of wild oats in both the field and greenhouse. Spray patterns achieved by the use of hollow cone and double fan nozzles increased the herbicidal activity of barban in the greenhouse but not under field conditions.

6. Although greatly reduced in numbers by all treatments, the wild oats seeds which did mature were found to be viable.

7. The application of phosphorus to a soil deficient in this nutrient enhanced the wild oats control with barban.

8. This study indicated the existence of different levels of tolerance between some varieties of wheat, barley, and flax. With wheat, the variety Selkirk was somewhat more susceptible than Thatcher at the 1½-leaf stage. All oats varieties tested were highly susceptible.

9. In general the action of barban tended toward reduction of growth and development of the wild oats rather than the actual killing of the plants.

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Penetration and Persistence of Diuron in Soil[1]

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The selective control of weeds in crops by the use of 3–(3,4–dichlorophenyl)–1,1–dimethylurea (diuron) has had considerable attention the last five years. Some of the problems of applying diuron are how long and at what depths the herbicide remains in the soil after treatment, with different methods and amounts of irrigation.

A study was undertaken with the following objectives: (1) To determine the penetration and persistence of diuron in soil after treatment of established alfalfa; (2) To evaluate the effect of different frequencies of irrigation on the penetration and persistence of diuron applied to the soil surface at various rates; (3) To determine whether retreatments of diuron penetrate and persist in the soil in the same manner as the original treatments; (4) To compare penetration and persistence of diuron in loamy sand and sandy clay loam soils.

Materials and Methods

Penetration and persistence of diuron in a sandy clay loam soil.

Diuron was applied July 16, 1957, at five rates on a field of established Ladak alfalfa at Laramie, Wyoming, immediately after removal of the first cutting of alfalfa hay. The treatments were replicated four times on plots 21 by 42 ft. The main plots were divided into subplots 21 ft. square. One subplot subsequently received minimal irrigation to maintain growth of alfalfa and the other was heavily irrigated for maximum hay production. Half of each subplot was retreated May 22, 1958, at the original rate. The area was flood-irrigated, whereby water was turned in on the upper side of the field and allowed to flow down the field over the entire surface and never impounded. Approximately 4 acre-inches of water were applied in each irrigation. The total number of irrigations applied to each irrigation block and the accumulated total precipitation prior to each sampling were recorded (Table 1). The 1958 retreatments were made before any of the 1958 irrigations.

Two soil samples were taken from each plot, 2 weeks, 3, 11, 13, and 15 months after the initial treatment. Each sample was subsampled by soil depths at 0 to ½, ½ to 2, 2 to 4, and 4 to 8 in. The two samples for each soil depth were thoroughly mixed to give a composite sample for each depth. Several important characteristics

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Table 1. Number of irrigations and total accumulated precipitation received before each sampling and the 1958 retreatments.

Treatment and sampling dates	Precipitation inches	Number of irrigations[a]		Total inches water received[b]	
		Light	Heavy	Light	Heavy
Treated—July 16, 1957	0				
July 29, 1957	1.65	0	1	1.65	5.65
October 21, 1957	5.07	1	4	9.07	21.07
Retreated—May 22, 1958	9.14	1	4	13.14	25.14
June 25, 1958	10.29	2	6	18.29	34.29
August 25, 1958	15.42	3	9	27.42	51.42
October 16, 1958	15.45	3	10	27.45	55.45

[a]Approximately 4 acre-inches of water were applied at each irrigation.
[b]Precipitation plus irrigation.

of the sandy clay loam soil at each sampled depth are described (Table 2). The samples were taken to the greenhouse for a bioassay with oats to determine the presence of diuron in the soil. The composite samples were used to fill a set number of pots for each sample date.

The same soil was treated with diuron to give known concentrations of 0.2 to 2.0 ppm. These were repeated with each sampling as standards by which to estimate the relative amount of diuron required to give a reduction in or elimination the oats. Samples of soil collected in the field containing lethal concentrations of diuron were not further diluted to give ppm of diuron in each sample.

Fifteen Overland oat seeds were planted in each pot and allowed to grow seven weeks. Then the surviving oat plants in each pot were counted, the plants clipped at the soil surface, and weighed green to the nearest decigram.

Persistence of diuron in a loamy sand soil.

Experiments were conducted near Riverton, Wyoming, for the control of annual weeds in established Ranger alfalfa during 1957 and 1958 (7). Diuron was applied at four rates April 13, 1957. Soil

Table 2. Important characteristics of soils at the two experimental locations.

Sample depth in.	Available phosphate lb/A	pH	Soluble salts E.C. millimhos	Organic matter per cent
Sandy clay loam at Laramie				
0 to ½	126	7.8	1.6	3.8
½ to 2	166	7.6	1.2	1.9
2 to 4	29	7.7	0.8	1.3
4 to 8	22	7.6	1.5	1.6
Loamy sand at Riverton				
0 to ½	47	7.5	1.4	4.7
½ to 2	26	8.0	1.2	1.2
2 to 4	7	7.6	0.6	0.3
4 to 6	7	7.5	0.6	0.8

samples were taken July 17, 1958, 15 months after treatment, from two replicates of each rate as described above, and bioassayed in the same manner. Soil samples were taken from the same depths as in the previous experiment except the fourth depth was from 4 to 6 in instead of 4 to 8 in. The nearest weather station, 4 miles from this field, reported 14.06 in of precipitation from April 13, 1957, until the soil samples were taken, July 17, 1958.

The experimental area was furrow-irrigated 2 to 4 times each growing season. The irrigations produced rather deep furrows between the 4-ft spaced alfalfa rows. All soil samples were taken from the row midway between the furrows. Irrigation water did not move over the surface of the sample areas, but the surface between furrows was moistened through capillarity.

A test was initiated March 29, 1958, on a nearby alfalfa field of the same soil type. Soil samples were taken from two replications of each of four rates on July 17, 1958, and bioassays conducted as above. A description of several important soil characteristics is found in Table 2. The weather station reported 5.53 in of precipitation for the 3½ months.

A second set of soil samples was taken 7 months after application of the diuron from plots treated at 2 lb/A. Samples were taken from the same depths as before and were air-dried in the greenhouse. However, a somewhat different bioassay technique was used. The soil was weighed and mixed with various amounts of nontreated soil from the same area to determine the approximate concentration of diuron in the original samples. These, when compared with soil containing known concentrations of diuron, indicated the approximate concentration of diuron present in the soil. Oats were planted as in the previously described method for the bioassay of diuron. In addition to the determination of the presence of diuron in each of the four depths of soil, a composite of all four depths was thoroughly mixed to simulate plowing, disking, and other field mixing of the surface 6 in of soil. This soil mixture was then diluted in the above manner to determine the concentration of diuron present in the soil.

RESULTS AND DISCUSSION

Penetration and persistence of diuron on a sandy clay loam soil.

Oats died at 0.4 ppmw and higher concentrations of diuron in soil from each of the samplings. Some reduction in growth resulted from lower concentrations. While the speed of oat kill was variable, the final results were always similar.

Bioassay of soil samples taken 2 weeks after treatment showed that diuron was present in the 0 to ½ in of soil from rates of ½ to 4 lb/A (data not shown). Diuron was present in the ½- to 2-in depth after treatment with 4 lb/A in both blocks. The 1.65 in of precipitation received during the 2-week period after the applications were made until sampling apparently was ample to move the herbicide as much as one irrigation plus rainfall.

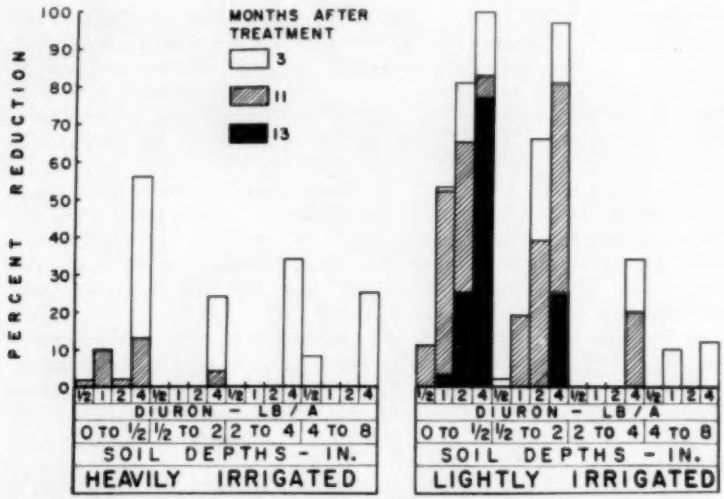

Figure 1. Reduction in growth of oats by diuron at different rates of application, sampling depths, and intervals after initial treatment. Values are expressed as percentage of fresh weight production of oats on untreated soil samples at each of the sampling depths, intervals after treatment, and under each intensity of irrigation.

Three months after treatment, diuron was present in significant amount in the heavily irrigated block only in the 0- to ½-in layer of soil treated at the 4-lb rate (Figure 1). Diuron was still present in the lightly irrigated block in the surface half inch of soil of the 1-, 2-, and 4-lb rates and in the ½- to 2-in layer of soil at the 2- and 4-lb rates. Eleven months after treatment diuron was no longer present in amounts phytotoxic to oats at any soil depth in the heavily irrigated block but was still present in the lightly irrigated block at the same depths and in only slightly reduced amounts from those present 3 months after treatment.

Bioassay of soil samples taken 13 months after treatment showed diuron at 4 lb/A was still present in the 0 to ½ in of soil. Diuron could not be detected in any of the other rates. Samples taken from the lightly irrigated block 15 months after treatment showed that diuron was no longer present in any of the treated areas.

The soil samples taken 11 months after the original treatments and 1 month after retreatment at the original rates showed considerable diuron present on most plots but two irrigations had removed the ½-lb rate completely from the soil in the heavily irrigated block (Figure 2). The depth of penetration of diuron and amount present varied directly with rate and was greater in the lightly irrigated block which had received only one irrigation from time of retreatment until sampling than on the heavily irrigated block which had received two 4-in irrigations.

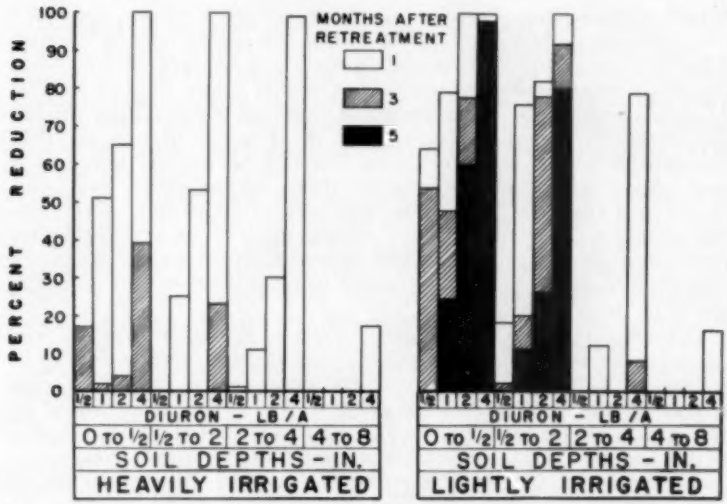

Figure 2. Reduction in growth of oats by diuron at different rates of application, sampling depths, and intervals after retreatment. Values are expressed as percentage of fresh weight production of oats on untreated soil samples at each of the sampling depths, intervals after retreatment, and under each intensity of irrigation.

Diuron was still present three months after retreatment followed by light irrigation in the 0 to ½ in of soil from plots retreated at the ½-, 1-, and 2-lb rates. The 2- and 4-lb rates were still present at the ½- to 2-in depth but had disappeared from the 2- to 4-in depth.

A small amount of diuron was present in the 0 to ½-in of soil 3 months after retreatment at the 4-lb rate followed by heavy irrigation, but none was detected 5 months afterward. The ½- and 1-lb rates applied twice had almost disappeared from the lightly irrigated soil 5 months after retreatment. Diuron was present in the surface 2 in of soil treated at the 2- and 4-lb rates. Diuron applied at rates of ½, 1, or 2 lb/A did not result in injury to alfalfa plants. The 4-lb rate caused some chlorosis of plants and retardation of growth. This was especially noticeable after the retreatments. Apparently, injury to alfalfa occurred only when diuron penetrated below the 2 in soil depth.

The results of the experiments on the sandy loam soil at Laramie show that there was considerable difference between light and heavy irrigation in the rapidity with which diuron disappeared from the soil. The disappearance of diuron from the soil appeared to be more a function of irrigation than of time. The 2-lb rate disappeared within 3 months under heavy irrigation, but 15 months were required for complete dissipation under light irrigation. The heavily irrigated block received four irrigations during the 3-month period

before diuron disappeared while 3 irrigations were applied over the 15-month period required for dissipation under light irrigation. This indicated that approximately the same amount of irrigation water was required for the removal of diuron under the two systems of irrigation used in the experiment. Natural precipitation during the 15-month period was only 15.42 in. The results indicate that the small amount of precipitation received during the test period had much less effect upon the penetration and persistence of diuron in the soil than did irrigation. The role of irrigation in the disappearance of diuron in a dry climate may be to provide better conditions for the growth of soil microorganisms and to maintain a larger amount of diuron in the soil solution than would normally occur in unirrigated soils.

Danielson (1), who studied the toxicity of monuron over a 2-year period in an area which received 97 in of precipitation and 23 in of irrigation, found no difference in the effect of irrigation and non-irrigation on the detoxification of monuron in soil as measured by the growth of indicator plants. It is probable that the high amount of precipitation resulted in ample movement of monuron to facilitate its breakdown by microbial activity and that the effect of the irrigation water was masked.

Diuron was leached relatively easily into the ½- to 2-in depth in the sandy clay loam at Laramie, though higher concentrations were always present in the 0- to ½-in layer. After retreatment the 4-lb rate was leached readily to a 4-in depth. The retreatments at Laramie appeared to dissipate from the soil at about the same rate as the original treatments. The lack of irrigation in the lightly irrigated block delayed disappearance but did not appear to aid in a build-up of diuron in soil. The results from the soil samples taken 3 months after the original treatments and 3 months after retreatment showed about the same trend, with diuron present in about the same depths. These findings were in close agreement with those of Hill, et al. (3) who found that monuron or diuron retreatments disappeared at about the same rate as the original treatments.

Persistence of diuron on a loamy sand soil.

Soil samplings made in July, 1958, on the loamy sand soil showed that at Riverton, Wyoming, 15 months after the treatments in April, 1957, all traces of diuron had disappeared from soil treated at the 1-lb rate but it was still present in significant amounts in the surface 2 in in plots that had received the 2- and 3-lb rates.

The soil samples taken 3½ months after the 1958 applications of diuron contained large quantities of diuron. Plant growth indicated that diuron was present in highly significant amounts in the surface 2 in of soil in plots that had received 1.5, 2.0, or 2.5 lb/A. The 5.53 in of precipitation which occurred from the time the applications were made until sampling in the 1958 experiment and 14.06 in in the 1957 experiment probably accounted for the leaching of diuron down 2 in in the loamy sand soil.

In soil samples taken 7 months after treatment with the 2-lb rate of diuron and diluted with various amounts of untreated soil it was found that there was approximately 1.8 ppmw of diuron present in the surface ½ in and 1.4 ppmw in the ½- to 2-in depth but none was present in the 2- to 4-in depth. When soil from all depths was thoroughly mixed, the resulting concentration of 0.4 ppmw diuron was not phytotoxic to oats.

The method of irrigation would undoubtedly have a large and determining effect upon the length of time diuron might be expected to persist in a soil in a dry climate. Under a semi-flood or shallow corrugate type of irrigation, water flows over the soil surface and has a percolating effect, thus providing for movement of diuron down into the soil. This was the case in the experiment conducted at Laramie on the sandy clay loam. At Riverton the water had to move by capillarity from deep furrows in the loamy sand soil. In the deep-furrow method of irrigation the movement of diuron in the soil may not have been affected by irrigation, and penetration into the soil may have depended solely upon precipitation. Sprinkle or flood irrigation would probably induce the greatest penetration of diuron into soil. Irrigation of alfalfa from deep furrows would probably result in the greatest conservation of diuron and longer control of the annual weeds germinating in the soil surface.

The 2-lb application to the loamy sand soil at Riverton would have been equal to approximately 3.0 ppmw of diuron in the surface 2 in immediately after application. It was found that 1.8 ppmw diuron persisted in the surface ½ in of soil and 1.4 ppmw in the ½- to 2-in depth 7 months after treatment. These two depths of soil mixed together would have had about 1.5 ppmw diuron. This would then have been a 50-percent reduction from 3.0 ppmw to 1.5 ppmw in the one growing season. Assuming this 50-percent reduction to be a normal loss, the concentration of diuron could never accumulate to 4 lb/A with repeated yearly applications of 2 lb/A.

It has been shown (7) that a single application of diuron will give weed control for a 2-year period at Riverton. The carry-over of 1.5 ppmw of diuron would be sufficient to explain the 2-year weed control by one application. If it were desired to plow the alfalfa 1 year after diuron had been applied, plowing 8 in deep and thorough mixing of the plowed soil should dilute diuron to 0.4 ppmw in the soil and minimize the effect of diuron on the succeeding crop.

Many workers (2, 4, 5, 6) have shown that organic-matter content of soil greatly affects the sorption and leaching of diuron and monuron. They are in close agreement that soils high in organic matter content allowed less leaching. Because of lack of tillage for several years in an established alfalfa field, a large amount of organic matter accumulates on the soil surface, with 2 to 4 times as much in the surface ½ in as in greater depths (Table 2). The extremely low moisture content of this layer of organic matter is not as conducive

to growth of soil microorganisms capable of degrading diuron as the soil of a much higher moisture content immediately below this layer. This large amount of organic matter probably accounts largely for the fact that higher concentrations of diuron remained at this depth throughout the experiments. The addition of irrigation water to the field would tend to leach the diuron out of this highly retentive organic layer on the surface down into the soil where higher moisture content would be more favorable to the activity of soil microorganisms. Diuron is also probably freer in the soil solution at greater depths because of the lesser amount of soil organic matter.

SUMMARY AND CONCLUSIONS

An experiment was initiated at Laramie, Wyoming, in 1957 to test the penetration and persistence of diuron in an established field of alfalfa growing on a sandy clay loam soil. Diuron was applied at five rates on each of two adjoining subplots, one of which was heavily irrigated and the other lightly irrigated by a semi-flood method. Ten months later half of each subplot was retreated with diuron at the original rate. Soil samples were taken from each plot from four depths of soil on five dates, and oats were planted for bioassay to determine the presence of diuron.

Soil samples were also taken 3, 7, and 15 months after applications of diuron on a loamy sand soil at Riverton, Wyoming, where irrigation was applied in rather deep furrows. Soil samples were taken and assayed as above.

The principal results of these experiments were as follows:

(1) Diuron applied once at 2 or 4 lb/A was not carried into the soil below the surface 4 in. Frequent irrigation increased the rapidity of diuron penetration into the soil but did not increase the final depth of penetration.

(2) Diuron was most heavily localized in the 0- to ½-in layer of soil with less in the ½- to 2-in depth. The leaching pattern and decomposition appeared to follow this same trend with the diuron lasting the longest in the 0- to ½-in layer.

(3) Frequent irrigation greatly increased the rate of disappearance of diuron from the soil.

(4) Diuron retreatments appeared to penetrate and persist in about the same manner as the original treatments except that the retreatment at 4 lb/A penetrated readily into the 2- to 4-in zone.

(5) Diuron at 2 lb/A disappeared in one growing season from the sandy clay loam soil under frequent semi-flood irrigation but persisted two growing seasons under less frequent irrigation.

(6) A 2-lb application of diuron remained in the surface 2 in of a loamy sand soil under deep-furrow irrigation for at least 15 months in amounts phytotoxic to oats.

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Post-Planting Weed Control in Garden Chrysanthemums[1]

R. E. Widmer and R. J. Stadtherr[2]

In a previous study Stadtherr and Widmer (1) reported that several herbicides provided excellent weed control in field plantings of garden chrysanthemums (*Chrysanthemum morifolium*). The present study was undertaken (a) to compare the best herbicides of the previous study with newer materials, (b) to obtain additional data on herbicides which had been tested for one year only, and (c) to determine the effectiveness of herbicides applied in granular form.

Materials and Methods

The weed control trials were conducted in plantings of garden chrysanthemums growing in a silt-loam soil in Horticulture plots on the St. Paul Campus of the University of Minnesota. The area used for the 1958 trials had been planted to chrysanthemums for approximately 25 years. It was infested primarily with purslane (*Portulaca oleracea*) and a limited population of dandelion (*Taraxacum officinale*) and scattered grasses. The area used in the 1959 trials was a different plot of land previously planted to squash. It was infested primarily with witchgrass (*Panicum capillare*), purslane, redroot pigweed (*Amaranthus retroflexus*), and prostrate pigweed (*Amaranthus graecizans*).

Plants of the variety Wanda which had been grown in 2½-in containers were planted in the field at a 2 × 3 ft spacing. The herbicides were applied to 3 × 8 ft plots, each containing four chrysanthemum plants. The plots were replicated four times each year.

In 1958, the chrysanthemums were planted in the field on June 16 and the herbicides applied on June 24. The following herbicides were applied in spray form (rates per acre): 0.25 lb 2–chloro–4, 6–bis (ethylamino)–*s*–triazine (simazine), 10 lb ethyl *N*,*N*–di–*n*–propylthiolcarbamate (EPTC), 0.8 lb 3–(3,4–dichlorophenyl)–1,1–dimethylurea (diuron), 0.4 lb diuron plus 3.6 lb sodium 2,4–dichlorophenoxyethyl sulfate (sesone), 0.4 lb diuron plus 3.7 lb 2,2–dichloropropionic acid (dalapon), 0.8 lb 3–(*p*–chlorophenyl)–1,1–dimethylurea (monuron), 3 and 8 lb isopropyl *N*–(3–chlorophenyl)carbamate (CIPC), 4 lb 2–chloro–*N*,*N*–diallylacetamide (CDAA), 2 lb 2–chloro–4–diethylamino–6–ethylamino–*s*–triazine (trietazine), 2 lb 2–chloro–4–diethylamino–6–isopropylamino–*s*–triazine (ipazine) and 2 and 6 lb tris (2,4–dichlorphenoxyethyl) phosphite (2,4–DEP). The following granular herbicides were also applied: 10 lb/A EPTC, 8 lb CIPC, 4 lb CDAA and 6 lb 2,4–DEP.

In 1959, the chrysanthemums were planted in the field on June 24 and the herbicides applied on July 1. Herbicides which were

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applied in spray form and which had not been used in the previous season included the following: 3 lb/A 1–*n*–butyl–3–(3,4–dichlorophenyl)–1–methylurea (neburon), 3 and 6 lb sodium salt of 2,3,6–trichlorophenylacetic acid (fenac), 3 lb 3–amino–2,5–dichlorobenzoic acid (amiben) 4 lb 2,5–dichloro–3–nitrobenzoic acid (M503), 2 lb *N*–3,4–dichlorophenyl)–2–methyl pentanamide (N4562), 4 lb dimethyl 2,3,5,6,–tetrachloroterephthalate (DAC893), 6 lb 2–chloroallyl diethyldithiocarbamate (CDEC) and 15 lb *O*–2,4–dichlorophenyl *O*–methyl isopropylphosphoramidothioate (M1329).

The following granular herbicides not used in the previous season were applied: 0.8 lb/A diuron, 3 lb neburon, 3 and 6 lb amiben and 15 lb *O*–2,4–dichlorophenyl *O*–methyl isopropylphosphoramidothioate (M1481).

All herbicides applied as sprays were put on in about 120 gal/A water at 30 psi using a knapsack sprayer with a flat spray nozzle. Herbicides in granular form were applied with a shaker can. The herbicides were directed so as to avoid contacting the chrysanthemums. The area was cultivated immediately before herbicide application. In 1958 the plots were irrigated one day prior to treatment, while showers provided moisture in 1959.

Counts of weeds in a 1 by 6 ft area in the center of each plot were used to evaluate the herbicides. The weed counts were made when the weed population in the most weedy treatments appeared to give excessive competition to the chrysanthemum plants. Weed control was considered excellent when the plots contained less than 1 weed per chrysanthemum plant and the weeds did not compete with the chrysanthemums; good when the plots contained 1 to 1½ weeds per chrysanthemum plant and the weeds gave the chrysanthemum plants limited competition only; and fair when the plots contained 2 to 4 weeds per chrysanthemum plant and the weeds provided moderate competition for the chrysanthemums. The chrysanthemum plants were not injured or retarded in growth by the herbicides except where indicated.

RESULTS

In the 1958 study the weeds were counted on August 13. The average population in the control plots consisted of 96% purslane and limited numbers of annual grasses, redroot pigweed, prostrate pigweed and dandelion. The weed counts as means of the four replications are given in Table 1.

Although all treatments significantly reduced the weed population, weeds were still present in great enough numbers to give the chrysanthemum plants excessive competition in plots which received spray applications of simazine, EPTC and CDAA, and granular applications of EPTC and CDAA. The most effective weed control was obtained with 8 lb/A CIPC liquid or granular and 2 lb ipazine. The chrysanthemum plants in three of the four replicates sprayed with 8 lb CIPC were 5 to 10% smaller than plants in other treatments. Good weed control was obtained with the following sprays: 0.8 lb/A diuron, 0.4 lb diuron plus 3.6 lb sesone, 0.8 lb monuron,

Table 1. Weed control obtained with post-planting applications of herbicides in garden chrysanthemums, 1958 and 1959.

Herbicide[a]	Rate lb/A	Weeds/16 sq ft			
		1958[b]	1959[c]		
			Total	Grasses	Broad-leaved weeds
Simazine	¾	135	—	—	—
EPTC	10	127	—	—	—
EPTC (gran)	10	73	—	—	—
Diuron	0.8	25	77	52	25
Diuron (gran)	0.8	—	77	66	11
Diuron + sesone	0.4 + 3.6	18	—	—	—
Diuron + dalapon	0.4 + 3.7	47	—	—	—
Neburon	3	—	69	58	11
Neburon (gran)	3	—	121	94	27
Monuron	0.8	20	—	—	—
CIPC	3	20	81	67	14
CIPC	8	6	—	—	—
CIPC (gran)	8	7	59	49	10
CDAA	4	116	105	87	18
CDAA (gran)	4	74	92	68	24
Trietazine	2	34	—	—	—
Ipazine	2	12	76	69	7
2,4–DEP	2	45	—	—	—
2,4–DEP	6	26	—	—	—
2,4–DEP (gran)	6	20	—	—	—
Fenac	3	—	7	3	4
Fenac	6	—	3	1	2
Amiben	3	—	76	56	13
Amiben (gran)	3	—	69	31	21
Amiben (gran)	6	—	52	64	12
M503	4	—	98	82	16
N4562	2	—	152	131	21
DAC893	4	—	82	59	23
CDEC	6	—	116	88	28
M1329	15	—	71	56	15
M1481 (gran)	15	—	49	40	9
Check		217	170	147	23
LSD 5% level		10	9		
LSD 1% level		14	12		

[a]Herbicides applied as spray unless otherwise indicated.
[b]Herbicides applied June 24; weeds counted August 13.
[c]Herbicides applied July 1; weeds counted August 17.

3 lb CIPC and 6 lb 2,4–DEP and 6 lb 2,4–DEP granular. Fair weed control was obtained with the following: 0.4 lb/A diuron plus 3.7 lb dalapon, 2 lb trietazine and 2 lb 2,4–DEP.

On June 24, 1958, 0.24 in of rain fell in the morning but it was clear in the afternoon when the herbicides were applied. Soil temp 2 in below the surface was 68°F. The mean temperature for the six-day period prior to application was 63° F and ppt totaled 0.25 in. The mean temp for the six-day period following June 24, was 70° F and ppt totaled 0.07 in.

In the 1959 study the weeds were counted on August 17. The average population in the control plots consisted of 85% witchgrass, 3% redroot pigweed, 5% prostrate pigweed, 4% purslane and 3% other weeds. Results are shown in Table 1. A breakdown of grasses and broad-leaved weeds as well as total weeds is given because of the relatively high count in most plots and because of the differ-

ence in ratio of grasses to broadleaved weeds from that of previous years.

All treatments significantly reduced the weed population but only fenac at 3 and 6 lb/A provided excellent weed control. Chrysanthemum plants in both treatments were a paler green in foliage color and reduced up to 20% in width and height and up to 40% in fullness of plant; the more severe symptoms occurring at the higher rate of application. Fair weed control was obtained with the following granular herbicides: 8 lb/A CIPC, 6 lb amiben and 15 lb M1481. Chrysanthemum plants in the 8 lb/A granular CIPC treatment were somewhat smaller in width than the plants in any other treatments except those treated with fenac.

On July 1, 1959, the weather was sunny and the soil temp 2 in below the surface was 67°F. The mean temp for the six-day period prior to July 1 was 65° F and ppt totaled 1.40 in. The mean temp for the six-day period following July 1 was 68° F and ppt totaled 4.02 in. The 4.02 in of rain fell on July 7 and although the plots were on relatively level ground, it caused some washing. Water was observed to have stood on parts of the area for as long as several hours.

DISCUSSION

CIPC and ipazine, which provided excellent weed control in 1958, were not as effective in the 1959 study. All treatments which were used both years were less effective in controlling weeds in 1959. Fenac, the only herbicide to provide excellent weed control in 1959, injured the chrysanthemums. Possible reasons for the variation in the results between the two seasons might include a difference in the ratio of grasses to broadleaved weeds in the two fields used, and the unusually heavy rainfall six days after application of the herbicides in 1959. As the breakdown of grasses to broadleaved weeds in Table 1 indicates that the herbicides were relatively ineffective on both groups, the heavy rainfall with the resultant leaching and washing would seem to be the likely cause for the relatively ineffective control.

The most effective post-planting herbicide applications reported previously (1) were 0.8 lb/A monuron, 0.4 lb monuron plus 3.6 sesone and 3.6 lb and 6 lb CIPC for 3 years; 4 lb CIPC plus 5.4 lb TCA for 2 years and 4 lb CDAA for 1 year. Monuron at 0.8 lb/A and CIPC at 3 and 8 lb gave similar results in 1958. CIPC at 8 lb/A, the only one of these three 1958 treatments repeated in 1959, was not as effective. CDAA at 4 lb/A did not provide satisfactory weed control in 1958 or 1959. Although the monuron plus sesone treatment was not repeated in 1958, a combination of diuron plus sesone was highly effective.

Stettmeier (2) reported that simazine was applied in the spring to chrysanthemums at 2–4 kg/ha (3.5 lb/A) without injury. Stadtherr and Widmer (1) reported that simazine at 0.5 lb/A and 1 lb controlled weeds effectively, but also injured the chrysanthemums. No injury was noted from simazine at 0.25 lb/A in 1958, but weed

control was poor. This variation in response might be accounted for by variation in plant variety, size, age, and stage of development, soil type or climatic conditions at the time of application.

Spray application of 8 lb/A CIPC reduced plant size 5 to 10% in 1958. Since weed control was almost as good at 3 lb/A, 8 lb in spray was eliminated in 1959. The suggested rate of 8 lb/A granular was used, however, and plant size was again reduced 5 to 10%. In the absence of any other symptoms, the effect of the higher rate of application of CIPC on plant size was not considered serious.

Similar results were obtained wth liquid and granular formulations of amiben, diuron, CIPC and 2,4–DEP. The liquid formulation was more effective with neburon and the granular more effective with EPTC, M1481, and CDAA. M1481, which uses fine vermiculite for the carrier was too light in weight for convenient application.

SUMMARY

1. Post-planting weed control studies in garden chrysanthmums were conducted in 1958 and 1959.

2. Excellent weed control was obtained in 1958 with 8 lb/A CIPC liquid or granular and 2 lb ipazine. Good weed control was obtained with 0.8 lb/A diuron, 0.4 lb diuron plus 3.6 lb sesone, 0.8 lb monuron, 3 lb CIPC and 6 lb 2,4–DEP and 6 lb 2,4–DEP granular.

3. The only herbicide to provide better than fair weed control in 1959 was fenac which also injured the chrysanthemums.

4. Liquid and granular formulations of amiben, diuron, CIPC and 2,4–DEP gave similar results. The liquid formulation was more effective with neburon and the granular more effective with EPTC, M1481 and CDAA.

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Some Chemical and Physical Properties of Weed Killers, Supplement 1[1]

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(The paper to which this is a supplement appeared in Weeds 8: 241–253. The following explanation of the table is repeated from that issue:

"The chemicals are arranged alphabetically according to Chemical Abstracts' nomenclature.

"Density is given at 20°C referred to water at 4°C. Specific gravities are at the indicated temperatures. The melting point is of the commercially used material unless otherwise specified. $-H_2O$ indicates loss of water.

"The pH value is for 5% solutions in water or alcohol unless otherwise indicated."

It is believed that the abbreviations, tabled in the previous paper, are clear without repetition. Ed.)

Sources of Information

1. Allied Chemical and Dye Corp., General Chemical Div. (Unpublished)
2. American Chemical Paint Company. (Unpublished)
3. Dept. of Agriculture, State of California, Robert Z. Rollins, Chief, Bureau of Chemistry. (Unpublished)
4. The Dow Chemical Company. (Trade booklets and unpublished)
5. E. I. du Pont de Nemours & Company, Inc. (Unpublished)
6. Geigy Agricultural Chemicals. (Unpublished)
7. Heyden Chemical Corporation. (Trade booklet)
8. Monsanto Chemical Company. (Unpublished)
9. Naugatuck Chemical. (Trade booklets)
10. Stauffer Chemical Company. (Unpublished)

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Name	Empirical formula	Sp. gr. or density °C	M.P. °C	Vapor pressure mm Hg @ °C	pH	Solubility		Remarks
						Water		
Acetamide, *N,N*-diallyl-2-chloro-,	$C_8H_{12}ClNO$			9 @ 121–123 $9.4X10^{-3}$ @ 20 $1.7X10^{-1}$ @ 50		1.97%	>50% in Alc. & Ketones. >20% in aromatic solv. & halohydrocarbons.	
Acetamide, *α*-(2,4-dichlorophenoxy)-,	$C_8H_7Cl_2NO_2$					.05% @ 25		
Acetic acid, chloro-,	$C_2H_3ClO_2$	1.58 20/20	61–63	760 @ 189.5		very sol.	org. solv.	deliq.
Acetic acid, 4-chloro-*o*-tolyloxy-, dimethyl amine salt	$C_{11}H_{16}ClNO_3$					sol.		needs sequ.
Acetic acid, 4-chloro-*o*-tolyloxy-, 2,2′-iminodiethanol salt	$C_{12}H_{18}ClNO_5$					sol.		needs sequ.
Acetic acid, 4-chloro-*o*-tolyloxy-, isopropanolamine salt	$C_{12}H_{18}ClNO_4$			low volt.		sol.	sl. sol. org. solv.	needs sequ.
Acetic acid, 2,4-dichlorophenoxy-, diisopropanolamine salt	$C_{14}H_{21}Cl_2NO_5$					sol.	sl. sol. org. solv.	needs sequ.
Acetic acid, 2,4-dichlorophenoxy-, isobutyl ester	$C_{12}H_{14}Cl_2O_3$					insol.	org. solv.	
Acetic acid, 2,4-dichlorophenoxy-, isooctyl ester	$C_{16}H_{22}Cl_2O_3$	1.152 20/20 1.145 25/4	<0	52.4 @ 264 30.71 @ 248	5.6	insol.	org. solv.	flash p = 340°F C.O.C. fire point = 415°F.
Acetic acid, 2,4-dichlorophenoxy-, methyl ester	$C_9H_7Cl_2O_3$	1.3706 37.8/4		volt.		insol.	sol. petr. solv.	
Acetic acid, 2,4-dichlorophenoxy-, polypropylene glycol ester	$C_8H_5Cl_2O_2\cdot(C_3H_7O)_2H$			low volt.		insol.	org. solv.	
Acetic acid, 2,4-dichlorophenoxy-, triisopropanolamine salt	$C_{17}H_{27}Cl_2NO_6$					sol.	sl. sol org. solv.	
Acetic acid, trichloro-, 3-(*p*-chlorophenyl)-1,1-dimethylurea salt	$C_{11}H_{11}Cl_4N_2O_2$		78–81		acidic	insol.	moderate in org. solv.	
Acetic acid, 2,4,5-trichlorophenoxy-, isobutyl ester	$C_{12}H_{13}Cl_3O_3$					insol.	org. solv.	

Acetic acid, 2,4,5-trichlorophenoxy-, iso-octyl ester	$C_{16}H_{21}Cl_3O_3$			low volt.		insol.	org. solv.	
Acetone, hexachloro-,	C_3Cl_6O	1.74 @ 20	−2	760 @ 204	7	sl. sol.	sol. alc. petr. & org. solv.	
Acrolein	C_3H_4O	.841	87.7			sol.	sol. alc., ether	unstable, inflam. liq.
Ammonium biborate, trihydrate	$NH_4HB_8O_7 \cdot 3H_2O$	2.38–2.95				7.27% @ 18		efflu., 16.43% B
Arsonic acid, methane-, disodium salt	$CH_3AsNa_2O_3 \cdot 6H_2O$					sol.	insol. alc.	12.85% sol. arsenic
Benzene, *o*-dichloro-,	$C_6H_4Cl_2$	1.303	−17.6	760 @ 179		insol.	very sol. org. solv.	
Benzoic acid, 2-(2,4-dichlorophenoxy) ethyl ester	$C_{16}H_{12}Cl_2O_3$					insol.	sol. org. solv.	
Benzoic acid, 2,5-dichloro-,	$C_7H_4Cl_2O_2$		155	volt. with steam 760 @ 301		sl. sol.	sol. org. solv.	
Benzoic acid, 2,3,5,6-tetrachloro-,	$C_7H_2Cl_4O_2$		180–182			sl. sol.	sol. org. solv. alc., acetone	
Benzoic acid, 2,3,5-trichloro-,	$C_7H_3Cl_3O_2$		162–163	not volt. with steam		sl. sol.	sol. org. solv.	
Benzoic acid, 2,3,6-trichloro-,	$C_7H_3Cl_3O_2$		87–99°C for mixed isomers. 126 pure	low volt.		sl. sol.	sol. org. solv. 42% methanol	stable to 60°C stable in light
Benzoic acid, 2,3,6-trichloro-, sodium salt	$C_7H_2Cl_3NaO_2$			low volt.		44% @ 25°C.		
Boric acid	H_3BO_3	1.435 @ 15	170 decomp. 185	non-volt. volt. in steam	5	5% @ 25	sol. alc. 22.2 g. per 100 ml. glycerin	17.50% B
Boron trioxide	B_2O_3	1.85	577	non-volt.		11,000 ppm	sol. alc.	31.07% B
Butyric acid, 4-(*p*-chlorophenoxy)-,	$C_{10}H_{11}ClO_3$		120–121			<1%	sol. alc. & acetone, insol. in xylene	
Butyric acid, 4-(4-chloro-*o*-tolyloxy)-,	$C_{11}H_{13}ClO_3$		101–102		4	<1%	>25% ethanol and other org. solv.	

Name	Empirical formula	Sp. gr. or density °C	M.P. °C	Vapor pressure mm Hg @ °C	pH	Solubility		Remarks
						Water		
Butyric acid, 4-(4-chloro-*o*-tolyloxy)-, 2-butoxy-1-methyl-ethyl ester	$C_{18}H_{27}ClO_4$					<1%	>25% ethanol sol. acetone	
Butyric acid, 4-(4-chloro-*o*-tolyloxy)-, x-butyl ester	$C_{14}H_{21}ClO_3$					<1%	>25% ethanol	
Butyric acid, 4-(4-chloro-*o*-tolyloxy)-, isoöctyl ester	$C_{19}H_{29}ClO_3$			low volt.		insol.	org. sol.	
Butyric acid, 4-(2,4-dichlorophenoxy)-,	$C_{10}H_{10}Cl_2O_3$		117-118 F.P. 119-120.5	low volt.	4.0	sl. sol.	org. solv., alc.	
Butyric acid, 4-(2,4-dichlorophenoxy)-, x-butyl ester	$C_{14}H_{18}Cl_2O_3$	1.17		low volt.		<1%	org. solv.	
Butyric acid, 4-(2,4-dichlorophenoxy)-, isoöctyl ester	$C_{18}H_{26}Cl_2O_3$			low volt.		<1%	org. solv.	unstable NaOH
Butyric acid, 4-(2,4-dichlorophenoxy)-, 2,2'-iminodiethanol salt	$C_{14}H_{21}Cl_2NO_5$			low volt.		sol.		
Butyric acid, 4-(3,4-dichlorophenoxy)-,	$C_{10}H_{10}Cl_2O_3$		111-2	low volt.		<1%	xylene, acetone, >10% alc.	
Butyric acid, 4-(3,4-dichlorophenoxy)-, x-butyl ester	$C_{14}H_{18}Cl_2O_3$					insol.	sol. org. sovl.	
Butyric acid, 4-(3,4-dichlorophenoxy)-, isoöctyl ester	$C_{18}H_{26}Cl_2O_3$			low volt.		insol.	sol. org. solv.	
Butyric acid, 4-(2,4,5-trichlorophenoxy)-,	$C_{10}H_9Cl_3O_3$		112-113 c.p. 110-112	low volt.		insol.	sol. org. solv.	
Butyric acid, 4-(2,4,5-trichlorophenoxy)-, *n*-butyl ester	$C_{14}H_{17}Cl_3O_3$					insol.	sol. org. solv.	
Butyric acid, 4-(2,4,5-trichlorophenoxy)-, isoöctyl ester	$C_{18}H_{25}Cl_3O_3$			low volt.		insol.	sol. org. solv.	
Butyric acid, 4-(2,4,5-trichlorophenoxy)-, dimethylamine salt	$C_{12}H_{16}Cl_3NO_3$			low volt.		sol.	insol. org. solv.	
Calcium metaborate	$Ca(BO_2)_2 \cdot 2\text{-}H_2O$		$-H_2O$ 200-300			4000 ppm @ 30°C	insol. org. solv.	

Carbamic acid, diethyl dithio-, 2-chloroallyl ester	$C_8H_{14}ClNS_2$			$2.2X10^{-3}$ @ 20 $2.4X10^{-2}$ @ 50		8.55	>50% in alc. and ketones. >20% org. sol.	
Carbamic acid, dipropylthiol-, ethyl ester	$C_9H_{19}NOS$	0.9543 @ 30		20 mm @ 127		sl. sol.	sol. org. solv.	
Carbamic acid, *N*-methyldithio-, sodium salt dihydrate	$C_2H_4NNaS_2 \cdot 2H_2O$				>7	41.9% 20°C	sl. sol. org. solv.	stable in conc. aqueous sol. decomp. in dilute water sol.
Carbanilic acid, *m*-chloro-, 4-chloro-2-butynyl ester	$C_{11}H_9Cl_2NO_2$		75	non-volt.	7.0	15 ppm @ 25°C		non-corrosive, non-hyg., non-flam.
Chlordane (1,2,4,5-6,7,8,8-octachloro-2,3,3a,4,7,7a-hexahydro-4,7-methanoindene)	$C_{10}H_6Cl_8$	1.59-1.63		2 mm @ 175		insol.	org. sol	
Ethanol, 2-(2,4-dichlorophenoxy)-, sulfate, sodium salt	$C_8H_7Cl_2NaO_5S$	1.70 @ 20	245 decomp.	0.1 @ 20	9.5	25.5		needs sequ., non-hyg., fire point 230°F.
Lead arsenate, dibasic	$PbHAsO_4$	5.786-5.930	decomp. >200 $-H_2O$ 280			insol.		
1,4-Naphthoquinone, 2,3-dichloro-	$C_{10}H_4Cl_2O_2$	1.70 @ 25	188° min. 192-193	2 mm @ 275		0.1 ppm	4% xylene 6% dioxane	subl., decomp. by alkalis, bulk density 4.5 lbs/gal.
Oxalic acid, bis(2-(2,4-dichlorophenol)-ethyl) ester	$C_{18}H_{14}Cl_4O_4$					insol.		
Phenol, 2-sec-butyl-4,6-dinitro-, 2,2'-2''-nitrilotriethanol salt	$C_{18}H_{27}N_3O_8$			low volt.		sol.	alc.	
Phenol, 2-sec-butyl-4,6-dinitro-, 2,2'-iminodiethanol salt	$C_{16}H_{22}N_2O_7$			low volt.		sol.	alc.	
Phenol, 2-sec-butyl-4,6-dinitro-, isopropanolamine salt	$C_{13}H_{21}N_3O_6$			low volt.		sol.	alc.	
Phenylmercuri tri-ethanol ammonium lactate	$C_{15}H_{25}HgNO_6$		126			sol.	alc. glycols sl. petr. oils	decomp. ultra violet light, ppt. halides & alk.
Phenylmercuri mono-ethanol ammonium acetate	$C_{10}H_{15}HgNO_3$					sol.	alc.	

Name	Empirical formula	Sp. gr. or density °C	M.P. °C	Vapor pressure mm Hg @ °C	pH	Solubility		Remarks
						Water		
Phosphoramidothioic acid, isopropyl-, *o*-(2,4-dichlorophenyl) *o*-methyl ester	$C_{10}H_{14}Cl_2NO_2PS$	1.28–55	51.36	low volt.	7.0	5 ppm	1–10% kerosene, 10–25% alc., xylene, acetone	pale yellow oil
Phosphorous acid, tris (2,4-dichlorophenoxy)- ethyl ester	$C_{26}H_{21}Cl_6O_6P$			non-volt.		insol.		
Phthalamic acid, *N*-1-naphthyl-, sodium salt	$C_{18}H_{12}NNaO_2$		240–245	non-volt.	7.5	sol.	insol. petr. solv.	
Phthalimide, *N*-1-naphthyl-,	$C_{18}H_{11}NO_2$		180	non-volt.		sl. sol.		
Propionic acid, 2,2-dichloro-, 2-(2,4,5-trichlorophenoxy) ethyl ester	$C_{11}H_9Cl_5O_3$	1.55 @ 50	40	low volt.	2.4–3.8	insol.	org. solv.	
Propionic acid, 2-(2,4-dichlorophenoxy)-,	$C_9H_8Cl_2O_3$		118–119			sl. sol.	org. solv., alc.	
Propionic acid, 2,2,3-trichloro-, sodium salt	$C_3H_2Cl_3NaO_2$		decomp. 240°C.			sol.	sol. alc, acetone	
Propionic acid, 2-(2,4,5-trichlorophenoxy)	$C_9H_7Cl_3O_3$		180–181			150 ppm	sol. alc, acetone	
Propionic acid, 2-(2,4,5-trichlorophenoxy)-, 2,2',2''-nitrilotriethanol salt	$C_{15}H_{22}Cl_2NO_6$		120.5–123	low volt.		sol.		
Sodium metaborate, anhydrous	$NaBO_2$ or $Na_2B_2O_4$	2.464	966	760 @ 1434		20% @ 20°C.	insol. petr. solv.	16.44% B
Sodium metaborate, octahydrate	$NaBO_2 \cdot 8H_2O$ or $Na_2B_2O_4 \cdot 8H_2O$						insol. petr. solv.	5.15% B or 7.85% B
Sodium octaborate, anhydrous	$Na_2B_8O_{13}$						insol. petr. solv.	25.42% B
Sodium octaborate, tetrahydrate	$Na_2B_8O_{13} \cdot 4H_2O$						insol. petr. solv.	20.98% B
Sodium pentaborate, pentahydrate	$Na_2B_{10}O_{16} \cdot 5H_2O$						insol. petr. solv.	21.63% B

Sodium pentaborate, tetrahydrate	$Na_2B_{10}O_{16}\cdot 4H_2O$						insol. petr. solv.	22.44% B
Sodium tetraborate, anhydrous	$Na_2B_4O_7$	2.367	741			1.2% @ 0°C.	insol. petr. solv.	21.50% B
Sodium tetraborate, pentahydrate	$Na_2B_4O_7\cdot 5H_2O$	1.815	$-H_2O$ @ 120			18% @ 62°C.	insol. petr. solv.	14.85% B
Stoddard solvent		0.784		distillation = <50% 350°F. end p. 410°F.				flash p. >100°F. T.C.T.
1,3,5,2H-Thiadiazine-2-thione, tetrahydro-3,5-dimethyl-,	$C_5H_{10}N_2S_2$		99.5 100–103 with decomp.	760 @ 37.8		sl. sol.	19.4% acetone, sol. $CHCl_3$ insol. ether and CCl_4	
s-Triazine, 2-chloro-4,6-bis(ethylamino)-,	$C_7H_{12}ClN_5$		104	low volt.		insol.	sl. sol. dioxane & ethyl-cellosolve	
s-Triazine, 2-chloro-4-ethylamino-6-isopropyl amino-,	$C_8H_{14}N_5Cl$		171–174	non-volt.		70 ppm	18,000 ppm methanol, 52,000 chloroform, 360 N-pentane, 25°C.	white crystalline
Urea, 1-*n*-butyl-3-(3,4-dichlorophenyl) methyl-,	$C_{12}H_{16}Cl_2N_2O$		101.5–103			5 ppm	sparingly sol.	stable neutral pH & normal temp. Hydrolyzes in acids, bases or elevated temp.
Urea, 1,3-bis(2,2,2-trichloro-1-hydroxy-ethyl)-,	$C_5H_8Cl_6N_2O_3$		191 with decomp.	low volt.		insol.	sol. cyclic ketones	

Effects of Different Densities of Downy Brome *(Bromus tectorum)* on Growth and Survival of Crested Wheatgrass *(Agropyron desertorum)* in the Greenhouse[1]

RAYMOND A. EVANS[2]

COMPETITION of downy brome (*Bromus tectorum* L.) with seeded perennial grasses is an important factor in the lack of success of many seedings on sagebrush conversion areas in the Great Basin. The seriousness of downy brome competition was emphasized by Stewart and Hull (7). Their work in Idaho indicated repeated failure in seeding of crested wheatgrass (*Agropyron cristatum* [L.] Gaertn.) on areas where downy brome was not eliminated or reduced. Hull and Pechanec (3) pointed out that many studies indicate that reduction of dense stands of downy brome is essential for the early establishment of seeded species. They also note that in some cases seeding of perennials has been successful without reduction of this weedy annual grass.

From greenhouse experiments, Rummell (6) showed that downy brome decreased establishment of seedlings of crested wheatgrass and western wheatgrass (*A. smithii* Rydb.). Rummell's study indicated that reduction in numbers of seedlings and of tillers, and weights of tops and roots of both perennial species resulted from downy brome competition.

The experiment reported here was planned (1) to determine the effects of different densities of downy brome on growth and survival of crested wheatgrass (*A. desertorum* [Fisch.] Schult.) during the period of germination, emergence, and growth of young plants, and (2) to assess the relative importance of soil moisture depletion and other factors in competition between the two species during this critical period.

PROCEDURE

The study was conducted in the greenhouse. Plants were grown in soil columns 3 feet high and a foot square contained in waterproofed wooden boxes. Holes were drilled in the bottom of the boxes and a layer of coarse gravel was placed in the bottom of each before the soil was added to insure that the soil would not be supersaturated. Soil for the experiment was collected from the first 8 inches on a typical sagebrush site. The soil was classified as Brown loam. Crested wheatgrass was seeded in each box in a single row at the rate of two live seeds per inch; an average of 18 seedlings were established per box. Plants of this species were grown alone and with 4, 16, 64, and 256 plants of downy brome per sq ft. Three replications were used. Seed of downy brome were planted at the

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same time as the crested wheatgrass. Downy brome seed were distributed evenly over the soil surface in each box and covered with about ¼ inch of soil. The growing period was 15 weeks, November 15, 1958 to February 28, 1959.

Minimum air temperature in the greenhouse was 50°F. and maximum ranged from 73° to 84° during the growing period. Soil temperatures in the boxes at 3 inches ranged from 52° to about 70°. Soil temperatures at 18 inches ranged from 52° to about 65°.

Soil in the boxes was saturated at the beginning of the study. Soil moisture was near saturation in all boxes at time of emergence. Light sprinkling was continued until the seedlings were established. No water was added after establishment.

Weekly soil-moisture measurements were made with Colman fiberglas soil-moisture units and a soil-moisture ohmmeter. The fiberglas units were placed in the soil at depths of 3, 6, 12, and 24 inches. Calibration of ohm readings of the moisture units in terms of moisture percent was done in the laboratory according to Kelley (4). Moisture percentages in terms of weight at specific bars of suction were determined by the pressure-plate and pressure-membrane methods (5).

Soil-moisture units with thermistors were installed in three boxes at each depth and soil temperatures were recorded weekly. Moisture readings in ohms resistance were corrected for temperature before the data were analyzed.

Shoot growth of the two species was sampled weekly by a modification of the height times ground cover method (HG) (2). A point-frame with 11 pins was set at 11 equal intervals across each box to sample the vegetation. The height of the hit and species hit were recorded for each of the 121 points. In this way, percentage ground cover and average height for each species were obtained. At the end of the 15-week growing period the shoot growth of each species was harvested, oven-dried, and weighed. The roots from one replication of each treatment were washed out, photographed, oven-dried, and weighed. Two replications were watered to check regrowth as a criterion of survival of the crested wheatgrass under the various treatments.

RESULTS AND DISCUSSION

Responses of species.

Shoot growth of crested wheatgrass as measured in terms of HG exhibited two distinct trends in relation to different densities of downy brome. The first was differential early growth and second differential ceasing of growth (Figure 1).

From the fifth to the eighth week an extreme reduction of shoot growth of crested wheatgrass occurred when grown with 64 and 256 downy brome plants per sq ft. Sixteen downy brome plants per sq ft prevented the accelerated growth rate from the eighth week which crested wheatgrass exhibited when grown alone. Four downy brome plants had no appreciable effect on the early growth of crested wheatgrass.

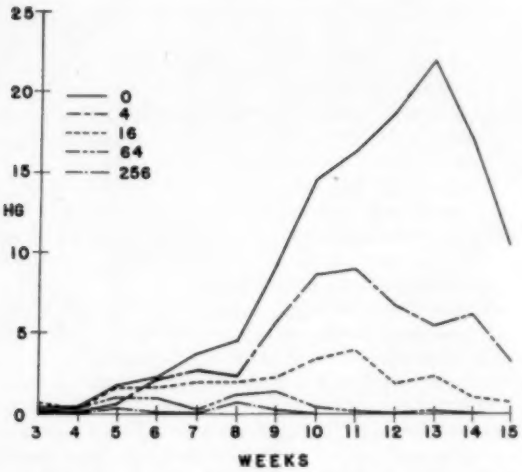

Figure 1. Shoot growth as measured by height times ground cover method (HG) of crested wheatgrass grown with 0, 4, 16, 64 and 256 plants of downy brome per square foot throughout the growing period.

From the eighth to the eleventh week differential cessation of growth of crested wheatgrass occurred in relation to densities of downy brome. With 256 downy brome plants per sq ft growth of crested wheatgrass ceased after eight weeks; with 64 plants after nine weeks; and with four and 16 plants after 11 weeks. When crested wheatgrass was grown alone growth ceased after 13 weeks.

Statistically significant differences occurred in maximum shoot growth of crested wheatgrass when the grass was grown (a) alone, (b) with 4 downy brome plants per sq ft and (c) with 16 to 256 downy brome plants per sq ft. No significant differences occurred in shoot growth of crested wheatgrass grown with densities of 16, 64, and 256 downy brome plants.

A trend of greater shoot growth of downy brome with higher densities was apparent at early stages of growth, but later these differences tended to decrease (Figure 2).

Differences in shoot growth of downy brome, as measured in terms of HG, occurred after three weeks of growth. At this time and until six weeks, shoot growth was greatest with 256 plants, next with 64, and least with 16 and four plants per sq ft. At six and seven weeks shoot growth in boxes having 256 and 64 plants was greater than in those having 16 and four plants per sq ft. At eight weeks shoot growth in boxes having 256 and 64 plants was greater than in those with four plants. At nine weeks shoot growth was significantly greater with 256, 64, and 16 plants than with four. At 10 weeks no significant differences existed in shoot growth among the different densities. Maximum shoot growth of downy brome

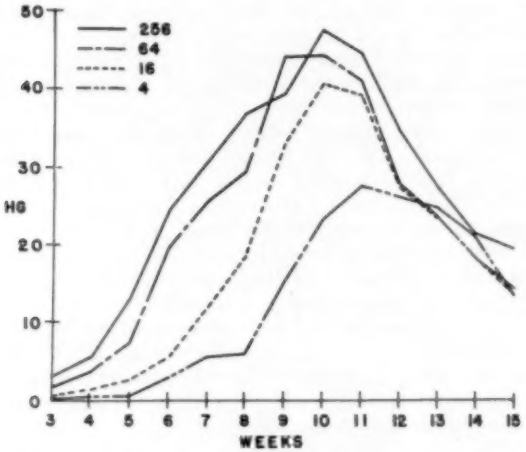

Figure 2. Shoot growth of downy brome as measured in terms of HG at 4, 16, 64 and 256 plants per square foot throughout the growing period.

was reached in 10 weeks at densities of 256, 64, and 16 plants per sq ft, and in 11 weeks at four plants per sq ft.

Shoot and root weights of crested wheatgrass at the end of the growing period were decreased when grown with downy brome (Table 1). Greater decreases were noted as the density of downy brome increased, especially when 64 and 256 downy brome plants per sq ft were grown with the crested wheatgrass. Shoot and root weights of downy brome were greater when 64 and 256 plants per sq ft were grown than when four and 16 plants per sq ft were grown.

Percent regrowth of crested wheatgrass, an indication of relative survival, decreased when it was grown with downy brome. Regrowth of crested wheatgrass when grown alone was 71%; when grown with four downy brome plants 64%; 16 plants, 66%; 64 plants, 20%; and 256 plants, 5%. These data indicate that chances for

Table 1. Root and shoot weights in grams of oven-dry material of crested wheatgrass and downy brome when grown together at different densities of downy brome.

Downy brome plants per sq ft	Crested wheatgrass		Downy brome	
	Shoot weight	Root weight	Shoot weight	Root weight
0	11.4 [a]	9.9	—	—
4	4.3	1.9	16.7	7.6
16	2.0	1.6	19.3	9.1
64	0.7	0.4	23.7	14.3
256	0.3	0.1	25.0	16.2

[a]Values opposite different lines are significantly different from each other at the 5% level of probability as determined by Duncan's multiple range test.

establishment of seedlings in the field under competition from the two latter densities would be very small. The effects of competition from 4 and 16 downy brome plants per sq ft were less severe. Increased chance for establishment of crested wheatgrass is indicated with reduced downy brome density.

When crested wheatgrass is seeded in an established and vigorously growing stand of downy brome, competition would be more deleterious for crested wheatgrass seedlings from any density of downy brome than has been indicated in this study.

In the field, downy brome will germinate and begin growth either in the fall or in the spring depending on the precipitation pattern. In years of early fall rains, seedlings of downy brome will begin to grow before the onset of low temperatures and thus provide severe conditions for the crested wheatgrass seedlings which normally germinate in the spring from a late fall seeding. On the other hand, in years of dry falls, seedlings of downy brome germinate about the same time in the spring as crested wheatgrass seedlings when the latter have been seeded in the fall.

Factors of competition.

Responses of crested wheatgrass indicate two distinct phases of competition with different densities of downy brome in relation to the growth period. The first is evidenced by reduction of shoot growth between the fifth and eighth week. The second resulted in cessation of growth at different times between the eighth and eleventh week.

Reduction of light for the crested wheatgrass seedlings in the first phase of the growth period was significa because of the closed canopy formed early by downy brome wi grown at high density. Consequently, least growth of crested wheatgrass during this first period occurred with the highest densities and greatest early shoot growth of downy brome (Figures 1 and 2). With 256 downy brome plants per sq ft crested wheatgrass plants grew practically from the beginning in conditions of reduced light. With lesser densities the shade factor was effective at a progressively later time, determined by shoot growth for a particular density.

Cessation of growth of crested wheatgrass at different times relative to densities of downy brome marked the second phase and was the result of depletion of soil moisture to 15 bars of suction at different rates.

Soil moisture was depleted more slowly when crested wheatgrass was grown alone than when downy brome at any density was grown with it. Soil at all depths was at 15 bars of suction after 13 weeks (Figure 3). This point coincides with the maximum growth of crested wheatgrass after which shoot growth ceased (Figure 1).

When 256 downy brome plants were grown with crested wheatgrass soil moisture was depleted to 15 bars in 9 weeks (Figure 4). Downy brome growth ceased after 10 weeks and crested wheatgrass growth ceased after 8 weeks (Figures 1 and 2). When 4, 16, and 64 downy brome plants were grown with crested wheatgrass soil mois-

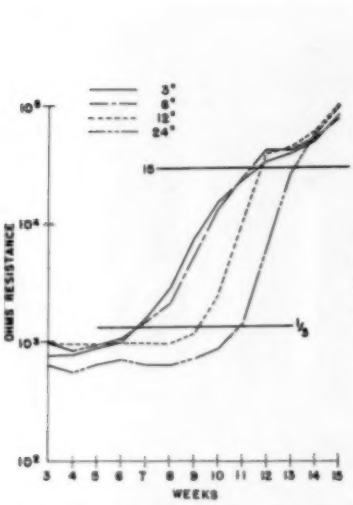

Figure 3. (Left) Soil-moisture depletion at 4 depths with crested wheatgrass grown alone. Lines indicate 1/3 and 15 bars of suction.

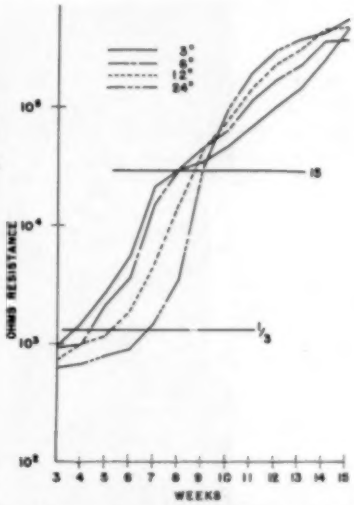

Figure 4. (Right) Soil-moisture depletion at 4 depths with crested wheatgrass grown with 256 downy brome plants per square foot.

ture was depleted to 15 bars in 12 to 10 weeks. Growth of both species ceased within a one-week range of when 15 bars of soil moisture suction was reached in these instances.

In general, the greater the density of downy brome the more rapid was the soil moisture depletion. This relation was true both in the early weeks of growth, depletion to 1/3 bar of suction, and in later weeks, depletion to 15 bars of suction and above.

An important factor in competition for soil moisture may be the difference in type and magnitude and in rate of growth of root systems of the two species. Downy brome possessed a more extensively branched root system with many fine rootlets, while the root system of crested wheatgrass was comprised of a large percentage of coarse roots (Figure 5). When the two species were grown alone in boxes with 16 plants of downy brome and 20 plants of crested wheatgrass the roots of downy brome weighed 22.9 grams whereas those of crested wheatgrass weighed 9.9 grams after a 15-week period (Table 1). More rapid soil moisture depletion early in the growth period occurred at 24 inches compared to 3 and 6 inches when downy brome was grown with crested wheatgrass than when crested wheatgrass was grown alone (Figures 3 and 4). This indicates, at least indirectly, a more rapid root growth of downy brome.

In the boxes used in the experiment, soil moisture was lowered from saturation mainly by the action of plants. Evaporation from the soil was of minor significance and then only in the top 6 inches.

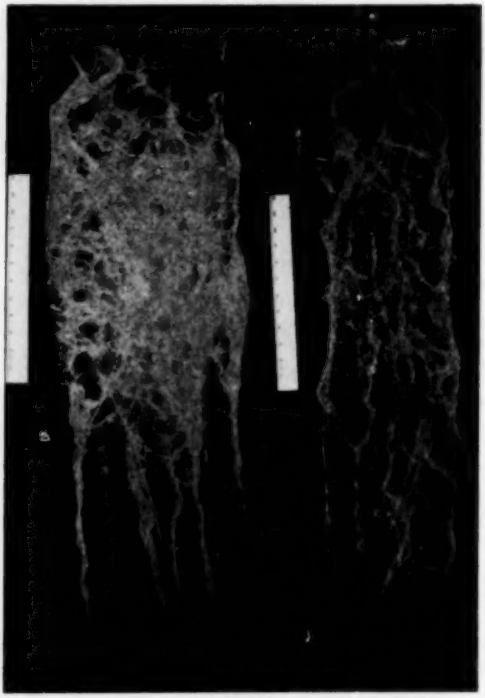

Figure 5. Root systems of downy brome with 16 plants (left) and of crested wheatgrass with 20 plants (right) grown for 15 weeks.

Drainage did not contribute to the lowering of soil moisture much below saturation.

Theoretically, the 3-foot soil column varied from approximately $^1/_{10}$ bar of suction at the top to saturation at the bottom as the result of drainage alone (1). To demonstrate these relations, soil in three boxes, prepared like the others, was saturated. One box had crested wheatgrass growing in it. The second with no plants was exposed to evaporation and the third was sealed at the top to prevent evaporation.

After a period of 15 weeks the soil in which crested wheatgrass was growing was well above 15 bars of suction. In the box where evaporation could take place soil at the 3- and 6-inch depths was near field capacity (⅓ bar of suction), while at 12 and 24 inches the soil was still saturated or nearly so. In the sealed box the soil was at or near saturation at all depths. Of course, with plant material covering the soil surface as in the experiment, evaporation would be less important than in the box with bare soil.

In well-drained soils in the field, soil moisture would be at or near field capacity (⅓ bar of suction) when air and soil temperatures became high enough to permit rapid growth of the two grasses in the spring. Therefore, in the absence of late spring rains soil moisture would become limiting much earlier between competing downy brome and crested wheatgrass in the field than it did in this experiment. In years of late spring rains soil moisture would become limiting later and the shade factor would be of more importance in competition between the two grasses.

SUMMARY

Effects of different densities of downy brome on growth and survival of crested wheatgrass during germination, emergence, and growth of young plants were studied in the greenhouse.

Downy brome at densities of 64 and 256 plants per sq ft severely curtailed shoot and root growth and greatly increased mortality of crested wheatgrass. At 4 and 16 plants per sq ft downy brome moderately decreased growth and survival of crested wheatgrass.

Shading of crested wheatgrass by downy brome was involved in the early phase of competition. Effects of shade competition were most severe at the two higher densities. Depletion of soil moisture to the permanent wilting point was the effective factor in the later phase of competition between the two species. Permanent wilting point was reached first with the higher densities of downy brome; next with the lower densities of downy brome; and last when crested wheatgrass was grown alone.

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Penetration of 3–Amino–1,2,4–triazole in Canada Thistle and Field Bindweed[1]

R. A. Herrett[2] and A. J. Linck[3]

The compound, 3–amino–1,2,4–triazole (amitrole), has been used as an herbicide against different weed species with varying degrees of success. In the field, Canada thistle (*Cirsium arvense* (L.) Scop.) has been found relatively susceptible (1,8) while field bindweed (*Convolvulus arvensis* L.) has been found relatively resistant (12) to the action of amitrole. The reason(s) for this difference in sensitivity is not known. Because penetration of amitrole from the surface of the leaf to the interior is the first step in the chain of events leading to a response from a chemical by the treated plant, the nature of this process was examined.

Methods and Materials

Both thistle and bindweed used in these experiments were vegetatively propagated from a single plant of each species and grown in 8-inch pots in light sandy soil. All applications of amitrole were made on plants in the vegetative stage (thistles 10–16 cm in height, bindweed 40–60 cm in length) in a water drop applied over the mid-vein of a single leaf approximately two-thirds the length of the leaf from the stem. The treated leaf was approximately at the mid-point on the stem.

Plants were allowed to grow and were treated with amitrole in either the greenhouse where light and temperature (75–95°F) varied widely or in controlled-environment rooms under artificial light (maximum 1000 f.c., supplied by incandescent and fluorescent lamps) having alternating 12-hour light (75°F) and dark (65°F) cycles. Thistle plants were sensitive to movement from the greenhouse to the controlled-environment room. This was indicated by the subsequent death of leaves present at the time of transfer and also, by the abnormal distribution of phosphorus-32 applied to the foliage following transfer[4]. To overcome this difficulty, Canada thistle plants were cut back to the soil level when moved from the greenhouse to the controlled-environment room. Bindweed did not appear to be sensitive to movement from the greenhouse to controlled-environment conditions. However, to be consistent, bindweed

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[4]Herrett, R. A. Studies on the absorption, translocation, and metabolism of 3–amino–1,2,4–triazole in perennial plants. Ph.D. Thesis, University of Minnesota. 1959.

plants were also cut back to the soil level when moved from the greenhouse to the controlled-environment rooms. One exception is stated below.

Quantitative analyses for amitrole were made utilizing modifications of the methods of Green and Feinstein (7) and Racussen (9). Briefly, a 3 ml sample obtained in the manner described below was tested for amitrole by placing it in an ice bath and $NaNO_2$ (0.5 ml, 1% aqueous solution), HCl (0.5 ml, 20% conc. HCl in water), and 1–amino–8–naphthol–3, 6–disulfonic acid (0.2 ml, 0.25% in 50% acetone-water) were added in that order. The solution was allowed to remain in the ice bath for 30 minutes to allow maximum color development. The absorbance of the sample at 538 mu was then measured with a Coleman Junior Spectrophotometer within 3 minutes after removal from the ice bath. The amount of amitrole was calculated from a standard curve which followed Beers-Lambert Law in the concentration range used (0.1-10 ugm/sample).

RESULTS

Effect of environment on the appearance of discoloration.

Discoloration refers to the development of new leaves lacking chlorophyll entirely or in part resulting in a whitish appearance of the affected organs. Amitrole was applied at various concentrations to duplicate thistle and bindweed plants growing in the controlled-environment room and plants growing in the greenhouse. The average time required for the appearance of discoloration in the two situations (Table 1) increased as the concentration of applied

Table 1. Time required for discoloration and for burning to appear after the application of amitrole to thistle and bindweed growing under controlled-environment room and greenhouse conditions.

Amitrole applied (ugm)	Hours required for appearance of symptoms after amitrole treatment							
	Thistle				Bindweed			
	Controlled-environment		Greenhouse		Controlled-environment		Greenhouse	
	Discolor.	Burn.	Discolor.	Burn.	Discolor.	Burn.	Discolor.	Burn.
1	—[a]	—	—	—	—	—	—	—
10	132	tr[b]	—	—	—	—	—	—
25	108	48	192	120	—	—	—	—
50	102	42	132	96	168	—	—	—
100	78	36	96	96	150	—	—	—
250	60	36	132	96	198	—	—	—
500	60	36	108	96	168	—	—	—

[a] — indicates no symptoms occurred.
[b] tr indicates a trace of burning at the point of application after 48 hours.

amitrole decreased. Thus, the sooner discoloration of the terminal apex appears, the more rapidly effective concentrations of amitrole reach the terminal apex.

Burning was characterized by the formation of a necrotic area (tissue brown, dry, and dead) approximately 200 mm^2 at the point

of application. The maximum area of the leaf covered by the applied drop was approximately 30 mm². Penetration of relatively large amounts of amitrole must have occurred to cause burning over such a large area. Considerably more time was required for discoloration and burning to appear with plants grown and treated in the greenhouse than those under controlled-environment conditions.

To determine if the difference in the appearance of discoloration between bindweed grown in the greenhouse and controlled-environment room treated with amitrole was a result of conditions before or after the application of amitrole, four bindweed plants growing in the greenhouse and four growing in the controlled-environment rooms were treated with amitrole (500 ugm). Immediately following treatment, two plants were moved from the greenhouse (without cutting back) to the controlled-environment room and two were moved from the controlled-environment room to the greenhouse. After 14 days, discoloration was apparent on bindweed grown in the controlled-environment room and placed in the greenhouse after treatment, but no discoloration was apparent on the plants grown in the greenhouse and moved to the controlled-environment room. This implied a pre-conditioning effect of environment on the response of bindweed to amitrole and further substantiated the necessity of cutting back plant material when transferred from one growing condition to another.

Effect of light on the appearance of discoloration.

To determine if light influenced the uptake of amitrole, 24 thistle plants were treated with amitrole (100 ugm) 6 hours after the beginning of the light cycle. Immediately following treatment, 12 plants were placed in the dark and 12 plants were allowed to remain in the light (1000 f.c.). The treated leaves of three plants from both the light and dark were washed at 0, 1.5, 3.0, and 4.5 hours after application. Nine days following treatment, no differences in plant response were observed between the uptake of amitrole in the light and in the dark.

To determine if the light period prior to treatment was important for uptake, the experiment was repeated in the same manner except that the plants were in darkness for the 12 hours before treatment. Again, no differences were found between plants which had absorbed amitrole either in the light or in the dark, indicating that the presence or absence of light either preceding or during the time period tested had no influence on penetration.

Effect of humidity on uptake.

To determine the effect of relative humidity on uptake, 48 thistle plants were treated with amitrole (20 ugm in 0.020 ml per plant) on the third leaf below the terminal apex. Twenty-four plants each were placed in two humidity conditions, one the controlled environment of approximately 35% and the other greater than 95%. The high humidity was obtained by placing the plants in a chamber containing a fogging machine. Identical light intensity was main-

Table 2. The influence of high and low humidity on the uptake of amitrole by thistle leaves.

Time of removal of amitrole from leaf after treatment	Average number discolored leaves	Average number of the leaf above the treated leaf showing symptoms
0	0	0
3 hr. high humidity	0	0
3 hr. low humidity	0	0
6 hr. high humidity	4	8
6 hr. low humidity	0	0
9 hr. high humidity	5	5
9 hr. high humidity	5	7

tained in each of the environments. The treated leaves were washed at 0, 3, 6, and 9 hours following application. Under controlled-environment conditions the amitrole solution applied to the leaves during the light cycle required between 45 and 90 minutes to dry visibly. Under high humidity, the drop of amitrole was visible on each leaf for from 6 to 9 hours. The observations of discoloration as well as the first leaf above the treated leaf to show discoloration were made after 9 days (Table 2).

Uptake under the high humidity condition resulted in increased responses at both 6 and 9 hour intervals, indicating improved penetration over that obtained under a low humidity. In the 6 hour uptake interval, no discoloration was observed under the low humidity whereas, it did appear under high humidity. In the 9 hour uptake interval, discoloration appeared under both the high and low humidity, but the high humidity again was more favorable as indicated by the lower number of the 'average number of the leaf above the treated leaf showing symptoms.' This indicates that an effective concentration of amitrole reached the terminal apex more rapidly under high humidity than under low humidity.

Quantitative estimation of amitrole absorbed.

The amount of amitrole absorbed was measured quantitatively by analyzing the residue on the leaf surface. This was done by washing the treated leaf three times with distilled water. The leaf washings were combined and brought to volume (50 ml) and an aliquot was analyzed directly for amitrole. Preliminary experiments showed the recovery from the surface of both bindweed and thistle leaves to be 100 ± 2% when the leaves were washed immediately following application. The uptake of amitrole applied 3 hours after the beginning of the light cycle to thistles and field bindweed grown in the controlled-environment room is shown in Table 3. Uptake for both thistle and bindweed is relatively constant through the period required for the droplet to dry (45 to 90 minutes). The rate of uptake per hour (per cent of the material applied) is of the same order for both plants (18.8% for thistle: 14.4% for bindweed). Upon drying, the rate of uptake per hour for bindweed (2.7%) is considerably less than that observed for thistle (7.6%). These figures

Table 3. Residue of amitrole remaining on treated leaves after various periods of time.

Time in minutes after application of amitrole when residue was removed	Average ugm amitrole recovered	Average pct. recovery	Rate of uptake per hour (pct. of the material applied)
Canada thistle (3 plants)			
0	246	—	—
10	232	94.6	32.4
60	213	86.7	13.3
90	206	84.0	10.7
120	176	71.8	14.1
720	50	20.1	7.6
Field bindweed (3 plants)			
0	211	—	—
10	206	97.6	14.4
75	172	82.0	14.4
720	142	67.9	2.7

include the uptake through drying period which is rather variable and the period after the applied droplet is completely dry. If uptake is constant for both species, as the data indicate, then the differences would be even greated for the period after the droplet had dried (6.0% for thistle; 1.3% for bindweed calculated from the 90 to 120 minute reading for thistle and from the 75 to 720 minute reading for bindweed).

Analyses of the residue amitrole on leaves of 3 bindweed plants grown in the greenhouse resulted in 86.4% recovery or a rate of uptake per hour of 1.2% of the material applied after 720 minutes. Thus, the previously observed difference in response between plants grown in the controlled-environment room and the greenhouse to amitrole could be explained, in part, by a reduction in uptake by the plants grown in the greenhouse.

Occurrence of stomata on the dorsal surface of thistle and bindweed leaves.

To determine if differences in the number of stomata between bindweed and thistle could account for the large difference in uptake, counts were made of the number of stomata present on the dorsal surface of five randomly selected areas of different leaves of two plants of each species. Canada thistle and field bindweed both had an average of 8.9 stomata on the dorsal surface per 0.38 mm^2 area.

DISCUSSION

The nature of the cuticle is markedly affected by environmental conditions[5]. Thus, if the cuticle is the primary portal, it would be expected that environment should greatly affect the penetration of the substances into a leaf. This was observed in the quantitative reduction of the penetration of amitrole and the increase in time

[5]Orgell, W. H. The isolation and permeability of plant cuticle. Ph.D. thesis, University of California. 1954.

required for discoloration and burning to appear (Table 1) on the leaves of greenhouse-grown plants compared to those in the controlled-environment rooms. These observations might be explained on the basis of cuticle size. Although such measurements were not made, it was noted that leaves of plants grown in the greenhouse tended to be more 'leathery' and much more rigid than leaves of similar age from plants grown under artificial light. Anderson (2) proposed that the limiting factor in the response of nutsedge (*Cyperus rotundus* L.) to amitrole was uptake. In the work presented here, uptake of amitrole by bindweed was found to be limited in comparison to the uptake by thistle (Table 3). However, uptake could not be considered the limiting factor in the distribution of amitrole in thistle when approximately 80% of the applied material was taken up in 12 hours (Table 3). Uptake under controlled-environment conditions (75°F 12-hour day and 65°F 12-hour night) was more rapid than in the greenhouse. The per cent uptake by bindweed grown in a greenhouse is comparable to results previously reported in beans (6) when it is considered that two different species were involved.

That the primary mode of uptake was through the cuticle was also indicated directly by the lack of the influence of light on uptake either before or after application of amitrole. Also, if penetration occurred through the stomata, it would be expected that the greater absorption of amitrole by thistles might be explained in part by the presence of more stomata in thistle than bindweed. Such was not the case, however. Weaver and DeRose (11) also concluded that stomata are not "important portals" of entry of 2,4–D (2, 4–dichlorophenoxyacetic acid) as an aqueous spray.

The role of penetration in determining herbicidal activity of 2,4–D remains uncertain. Ashton (3) found relatively little difference in penetration between resistant and susceptible species. On the other hand, Fang and Butts (5) observed considerable differences between susceptible bean plants and resistant monocotyledons and Fang (4) reported differences between peas and tomatoes in their ability to continuously absorb 2, 4–D. In the present study, the rate of uptake by both a susceptible and resistant species appeared to be nearly the same as long as the amitrole remained in solution. However, once the droplet had dried, the rate of uptake by the sensitive thistle plant was greater than that observed for the resistant bindweed. In both cases, the rate of uptake was substantially less, once the droplet dried. Increasing the humidity during the uptake period probably increased absorption by increasing the time required for the droplet to dry. This would support the suggestion of van Overbeek (10) that hydration of the cuticle spreads the cracks in the wax allowing increased penetration.

Thus, there is a major difference in the uptake of amitrole between thistle and bindweed. This occurs after the solution has dried. The cuticle, which can be modified under different environmental conditions, is the most likely factor responsible for this difference as it appears to be the primary means by which penetration into

the leaf occurs. It is not proposed, however, that penetration is the only reason for the difference between thistle and bindweed in their sensitivity to amitrole.

SUMMARY

The penetration of amitrole into a resistant species, field bindweed, and a susceptible species, Canada thistle, was studied in an attempt to clarify the nature of this difference in sensitivity. It was found that:

(1) Penetration was primarily cuticular.
(2) Penetration of amitrole from solution occurred rapidly and at similar rates for both thistle and bindweed.
(3) Penetration of amitrole, once the solution applied to the leaf had dried, was much slower than when in solution but was much more efficient in the case of the sensitive thistle than the resistant bindweed.

It was proposed that differences in the uptake of amitrole between thistle and bindweed account in part for the difference in sensitivity between the two species.

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The Uptake, Translocation and Metabolism of Simazine and Atrazine by Corn Plants[1]

MARVIN MONTGOMERY and VIRGIL H. FREED[2]

INTRODUCTION

A NUMBER of the substituted symmetrical triazines have been shown to possess considerable herbicidal activity (2,3). Their high biological effectiveness against a wide spectrum of plants has aroused much interest in their use. While they have been found useful as soil sterilants, they are particularly valuable as selective herbicides on a number of crops. Two of the more promising triazines are simazine (2–chloro–4,6–bis(ethylamino)–*s*–triazine) and atrazine (2–chloro–4–ethylamino–6–isopropylamino–*s*–triazine). Both of these compounds have found extensive use in the control of weeds in corn. For this reason, it was of interest to determine the absorption, translocation and metabolism of these compounds by corn plants. The studies of this nature reported here were undertaken using C^{14} ring-labeled triazines. The labeling of the triazine was accomplished using C^{14} tagged cyanogen chloride in the synthesis, thus giving a random labeled ring.

METHODS AND MATERIALS

Treatment was accomplished by dissolving the C^{14} labeled triazine in chloroform and mixing aliquots of this solution with a small amount of soil to give the desired rate of application. The treated soil was dried and mixed thoroughly. Containers were filled with Chehalis sandy loam and planted with corn (var. Tendermost). The treated soil was distributed evenly over the planted containers to a depth of one-quarter inch and water was added to bring the soil to about field capacity and added thereafter as needed. The application was made at the rate of 0, 2 and 8 pounds of herbicide per acre.

The plants were harvested periodically up to and including maturity of the ear. Upon harvesting, one sample of plant was dried and the total amount of radioactivity in the dried tissue was determined. The radioactivity was determined using a thin window (1.9 mg/cm^2) GM tube. The tissue was dried and ground, placed in a planchet and the C^{14} counted. All counts were corrected for background and self-absorption. Another sample of green plant material was macerated and exhaustively extracted with chloroform in order to determine the radioactivity in this fraction. In this case, aliquots of the chloroform extract were plated and counted. Again counts were corrected for background counts and self-absorption. Previous work had shown that the triazine herbicide could be extracted

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quantitatively from plant tissue with chloroform in a Soxhlet extractor. The chloroform soluble radioactivity represents the maximum possible amount of triazine.

Paper chromatography was used in determining whether simazine as such was present in the chloroform extract of simazine treated plants. The solvent systems, consisting of either 65% 2,6–lutidine: 35% water, v/v, 50% 1–4 dioxane: 50% water, v/v, or 85% acetonitrile:15% water, v/v, were used to develop the chromatograms on Whatman number one paper. In order to ascertain whether any intact triazine was present in the chloroform extract of atrazine treated plants, ion exchange resins were employed. Paper chromatography was not feasible because of the low specific activity of the labeled atrazine. Atrazine was found to be strongly absorbed from a chloroform solution by a dry Dowex–50 X–8 cation exchange resin in the hydrogen form. Conversely, no atrazine is absorbed by passing a chloroform solution through a Dowex 1 column.

A further approach ascertaining the fate of triazines in corn plants, was an experiment carried out to give direct evidence as to whether corn plants are able to metabolize these compounds as indicated by the evolution of $C^{14}O_2$. Corn seedlings four to eight inches tall, grown in sterile sand, were transferred to an aqueous solution of radioactive triazine and placed in an airtight chamber. The carbon dioxide expelled by the plant was drawn off and collected as barium carbonate. At the end of the experiment, the plants were dried and the radioactivity in the dried tissue and the barium carbonate was determined. Radioactivity found in the carbonate indicates cleavage of the triazine ring since the labeled carbon atoms are in the ring. Tests with the solution both before and after corn seedlings had been in it showed that the $C^{14}O_2$ evolved was due to the presence of the plant.

RESULTS AND DISCUSSION

It was found that appreciable amounts of C^{14} labeled herbicide were taken up by corn from soil treated with either simazine or atrazine. These plants were harvested, dried, ground and 100 mg plated in a planchet. The concentration of radioactivity calculated as the respective triazine after correction for self-absorption is presented in Table 1. It should be noted that the times of harvest for the two compounds are slightly different both in this table and in Table 2. This arises from the fact that the experiments with the two compounds were conducted at different times.

It should be recognized that even though the triazine may be metabolized by the plant, the C^{14} may be incorporated into plant constituents and thus still appear in the plant. However, in this table, the radioactivity is expressed as if it were all in the form of the respective triazine.

It is interesting to note from this table that the pattern of uptake for both herbicides is nearly the same. The concentration of C^{14} labeled herbicide(s) in the plant early reaches a maximum and then

Table 1. Uptake of C^{14} by corn plants growing in soil treated with radioactive simazine and atrazine.

Rate lb/A	Simazine		Atrazine	
	Days following treatment	C^{14} expressed as ppm simazine, green wt.	Days following treatment	C^{14} expressed as ppm atrazine, green wt.
2	14	3.5	21	4.8
8		8.2		19.0
2	30	3.5	56	2.5
8		15.6		17.6
2	49	3.1	113	1.01
8		13.0		11.0
2	91	2.9	113, ears	.17
8		10.8		.62
2	119	2.2		
8		10.1		
2	119, ears	.3		
8		1.2		

undergoes a continuous decline. Although the decline may be attributed in part to the dilution due to growth, the plant is either taking up less herbicide or metabolizing it faster than it is being taken up. Subsequent studies indicate that metabolism is probably the dominant factor in this decline.

Attempts were made to determine what fraction of the C^{14} could be in the form of the original triazine. For this purpose, the tissues was extracted exhaustively with chloroform in a Soxhlet extractor and the radioactivity in the extract was determined. The percentages of the total C^{14}-containing chemicals which were soluble in chloroform are given in Table 2.

The radioactivity found in the chloroform extract is expressed as if it were all in the form of the parent triazine. This is only for purposes of convenience in estimating concentrations and consequently the reader is warned from inferring that all of the radioactivity is due to the unchanged triazine.

Table 2. The percentage of total C^{14} in treated corn which was chloroform extractable.

Rate lb/A	Simazine		Atrazine	
	Days following treatment	Pct. C^{14} chloroform extractable	Days following treatment	Pct. C^{14} chloroform extractable
2	49	41.0	21	83
8		42.4		89
2	91	33.8	56	75.4
8		39.8		71.2
2	119	18.1	113	72.3
8		28.7		54.9
2	119, ears	53.8	113, ears	48.8
8		39.4		59.6

It is apparent from the data, that just as the concentration of C^{14} diminishes in time, so does the percentage which is chloroform soluble. However, the percentage of C^{14} which is chloroform soluble does not reach a maximum but declines steadily from the first time of harvest. This strongly suggests that the corn plant is breaking the triazines down. In attempting to determine whether simazine was present in the treated plants, the chloroform extracts were chromatographed with three different solvent systems using descending chromatography (1). The first solvent system used was 2,6–lutidine: water. With this system simazine, alone, had an R_f of .85 to .92, while that of simazine, added to untreated plant extract, had an R_f of .81 to .90. With the treated plant extract, 70% of the radioactivity was below R_f of .80. The second solvent system was 1,4–dioxane:water. In this system, simazine added to untreated extract had an R_f of .83 to .94. In this case about 90% of the radioactivity was below R_f of .83. Results with the solvent system acetonitrile: water, were similar in that most of the radioactivity was spread out over a range which falls below that at which simazine is found. The range of R_f values was due to the low level of radioactivity which made it necessary to use a large amount of extract for the radioactivity to be detectable. However, from the spread of radioactivity in the chromatogram, there appeared to be more than one component containing C^{14} in the range below which simazine is found, indicating metabolism of the triazine ring and incorporation of the C^{14} into other compounds.

In an attempt to obtain complete separation, the chloroform extract of treated corn was developed with 50% dioxane. With this solvent, a considerable amount of the plant material was left at the origin while the radioactivity moved down the paper. The paper below the origin was then exhaustively extracted with chloroform and this extract was developed with 65% 2,6–lutidine. The range of R_f was .69 to .82 but was extended to .92 when radioactive simazine was added. This was interpreted as indicating that no simazin was present in the extract.

In attempting to determine whether atrazine was present in the chloroform extract of treated plants, the use of paper chromatography was precluded as the radioactivity was too low to be measured by a chromatogram scanner. Consideration of the problems of fractionating the low level of radioactivity led to the suggestion that this could be effected by the use of ion exchange resins. It was found that atrazine was strongly adsorbed by a Dowex–50 ion exchange column and no adsorption occurred on a Dowex–1 column. Subsequently, fractionation was achieved by passing five millimeters of solution through a column of Dowex-50 and the radioactivity in an aliquot of effluent was measured. Table 3 presents the information obtained upon passing an atrazine solution and the chloroform extracts of the plant from final harvest through the Dowex–50 column.

It should be noted in this table that the C^{14} extracted from the leaves and ears of the treated plants behaved substantially differ-

Table 3. Adsorption of C^{14} of atrazine or chloroform extracts of plants by Dowex–1.

Solution	Atrazine rate/A	Days following treatment	cpm/0.5 ml		Per cent retained
			Before	After	
Atrazine	–	—	374	3.3	99.1
Plant extract	2	119	22.7	6.3	72.2
Plant extract	8	119	207.7	28.1	86.5
Extract of ear	8	119	61.8	32.0	48.2

ently from the atrazine alone. This indicates the C^{14} is probably in some chemical form other than a triazine ring, suggesting that metabolism of the triazine has occurred. In order to make further investigations of the nature of the compound or compounds containing C^{14}, the extract was passed through a Dowex–1 column.

Inasmuch as the atrazine is not adsorbed on this type of resin, any radioactivity retained by this column must be something other than atrazine. Table 4 presents the information of the adsorption of C^{14} by a Dowex–1 column using the extract of plants from the second and final time of harvest.

Table 4. Adsorption of C^{14} of atrazine or chloroform extracts of plants by Dowex–50 column.

Solution	Atrazine rate/A	Days following treatment	Per cent retained
Atrazine	–	—	0
Plant extract	8	56	56.2
Plant extract	8	119	78.1

It should be pointed out in connection with this table that the final harvest is the most significant one in regard to a residue problem since this is approaching the normal time of harvest. In this instance, it is seen that in the final harvest 78% of the radioactivity is retained on the column in contrast to atrazine where no radioactivity is retained. In order to determine whether any of the radioactivity in the effluent of a Dowex–1 column behaved differently from atrazine, the effluent of a Dowex–1 column was subsequently passed through a Dowex–50 column. Of the 21.9% not retained by the Dowex–1 resin, 16.1% passed through the Dowex–50 resin and, therefore, was not atrazine. Thus, it may be seen that nearly 82% of the radioactivity was in a form other than as atrazine. This, then, is a very clear indication of an extensive degradation of this compound by corn plants.

To illustrate the level of radioactivity under consideration, this 18% of the chloroform extractable radioactivity which could be in the form of atrazine was calculated in terms of parts per million atrazine in the corn plant. Using this value, the maximum amount of atrazine in the ear in the two pounds per acre treatment would be only .02 parts per million and in the leaves .04 parts per million.

The results of the measurements of the uptake and solubility of radioactivity with time and the behavior of the chloroform extract on paper chromatogram and ion exchange resins indicates extensive degradation. However, the most conclusive demonstration of the ability of corn plants to degrade these triazines came from the experiments in which corn seedlings were placed in an aqueous solution of triazine in an airtight system similar to that described by Tokanskaya (4). Carbon dioxide-free air was drawn through the system and passed through CO_2 free sodium hydroxide to trap any carbon dioxide given off by the plants. The radioactivity found in the carbonate clearly demonstrates the ability of the corn plant to degrade the triazine nucleus since the C^{14} label is in triazine ring. In a similar experiment with radioactive triazine solution in the absence of corn, no detectable volatilization of C^{14} labeled chemical was found to occur. The results of these experiments are presented in Table 5. In the first two experiments the plants were exposed

Table 5. Metabolism of C^{14} simazine and atrazine by corn plants as measured by the release of $C^{14}O_2$.

Experiment	Duration hours	μg triazine metabolized	μg triazine remaining in plant	Per cent triazine metabolized of that taken up
Simazine 1	50	.48		
2	48	.19	2.0	8.7
3 (in dark)	68	.91	.53	63.2
Atrazine 1	54	.67	3.05	18.0
2	72	.30	1.49	16.8
3 (in dark)	70	.28	.73	27.7

to the normal daylight and darkness periods while in the third they were kept in total darkness for the entire experiment.

These results clearly demonstrate cleavage of the triazine ring and metabolism of the products. It is of significance that, as would be expected, a much larger percentage of the triazine taken up is metabolized when the plants are grown in the dark for the entire experiment.

SUMMARY AND CONCLUSIONS

Corn plants were grown in soil treated with two and eight pounds per acre of C^{14} labeled simazine or atrazine. The total amount of C^{14} in the plants and the percentage which could be in the form of the respective triazine was determined. It was found that the concentration of total C^{14} in the plants decreased after about 30 days and that the percentage which was chloroform extractable steadily decreased from the first time of harvest. This in itself, strongly suggests that these compounds are metabolized. The evidence for metabolism was confirmed by means of ion exchange and paper chromatography where it was shown that only trace amounts, if any, of these materials remained unchanged in the plant. This

information, coupled with the demonstration that the corn plant is able to degrade these triazines to $C^{14}O_2$, leads to the conclusion that the corn plant readily metabolizes these compounds.

Note: The authors wish to dedicate this paper to the memory of C. C. Alexander whose interest and suggestions in this work proved so stimulating.

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Control of *Pennisetum clandestinum*, Kikuyugrass[1]

VICTOR B. YOUNGNER and J. R. GOODIN[2]

Pennisetum clandestinum Hochst. is a vigorous fast growing grass introduced into sub-tropical areas of the United States from tropical Africa in approximately 1920 (5, 7). It has been used for soil erosion control and turf to a limited extent in California. However, in many places it has become a serious weed.

Kikuyugrass is a valuable forage grass in many tropical and subtropical regions of the world (2,3,8). Its aggressiveness and dense growth habit have made it a useful species for reclaiming pasture and range areas over-run by species of low forage value (9). Edwards (1), and Narayan (4) have described distinct male sterile and male fertile types. Robbins et al. (6) report that seed and seedlings have been observed in Orange County, California.

Control and eradication of kikuyugrass is difficult to accomplish. Hosaka (2) reports successful eradication in Hawaii by cultivation and by the herbicides TCA, monuron, and dalapon. This paper reports results of four years of study of control problems and control methods in southern California with principal reference to turf areas where kikuyu is an especially severe problem.

Kikuyu is often confused with St. Augustinegrass, *Stenotaphrum secundatum* (Walt) Kuntze, and has been frequently planted as turf by mistake. However, it resembles St. Augustinegrass only superficially in having a wide blade and heavy stolons. Kikuyugrass has a heavily pubescent, long, tapering blade; pubescent sheath and a round shoot in cross section. St. Augustinegrass has glabrous blades which are parallel-sided with a rounded tip. The shoots are greatly compressed laterally.

MATERIALS AND METHODS

This problem was attacked in two ways, first to determine methods of eradication and second to study natural dissemination and possible techniques for preventing spread from established stands.

Chemical control methods were evaluated on established turfs primarily consisting of *P. clandestinum*. The areas used were adequately watered but were low in nitrogen content. Thirty-three herbicides were used alone and in various combinations at several rates and in one and two applications. Screening tests consisted of 50 sq ft plots, replicated twice in a randomized block design.

Herbicides showing promise in screening trials were studied further in larger scale field tests in several locations. The following herbicides were tested in field trials: sodium–N–methyldithiocarbamate (SMDC), 2,2,–dichloropropionic acid (dalapon), 1,2–dihydropyridazine–3,6–dione (MH),3–amino–1,2,4–triazole (amitrole), 2–(2,-

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4,5–trichlorophenoxy)ethyl 2,2–dichloropropionate (erbon), methyl bromide, and 2–chloro–4,6–bis(ethylamino)–*s*–triazine (simazine). The following herbicide mixtures or combinations were also used: sodium chlorate, 40%, sodium metaborate, 58%; dalapon, 50.8%, 2–(2,4,5–trichlorophenoxy) propionic acid (silvex), 7.7%; amitrole, 16 lb/A, simazine, 2 lb/A; amitrole, 16 lb/A, ethyl *N*,*N*–di–*n*–propylthiolcarbamate (EPTC), 6 lb/A; and dalapon, 5 lb/A, amitrole, 16 lb/A. Effectiveness was determined by rating plots for initial kill, amount of regrowth and amount of new seedling growth.

Four-inch plugs of established kikuyugrass turf were grown in pots in the greenhouse to study the effects of five growth regulating chemicals on flowering. Growth regulators tested were: MH; 1–naphthaleneacetic acid (NAA); (4–hydroxy–5–isopropyl–2–methylphenyl)trimethylammonium chloride, 1–piperdine carboxylate (Amo–1618); tris(2,4–dichlorophenoxyethyl) phosphite (2,4–DEP); and 2,4,5–trichlorophenoxyacetic acid (2,4,5–T). Four replications of three concentrations of each chemical were arranged in a randomized block design. Each pot was sprayed with approximately 2 ml of solution at weekly intervals using an atomizer. Restriction of flowering by the chemicals was recorded as the total number of flowers produced during the experiment. As flowers lasted for at least two days, counts were made every other day. Plants were studied in the field and greenhouse for growth and morphological characteristics important to the development of control measures.

RESULTS AND DISCUSSION

Field and greenhouse studies showed that kikuyugrass grows throughout the year in climates where freezing temperatures seldom occur. It is highly drought tolerant, surviving the long, dry, California summers. However, it becomes a serious weed only on irrigated lands. Once established in such areas, it spreads rapidly by both stolons and rhizomes.

The fertile and sterile strains are both common in southern California. The male sterile strain is female fertile and thus produces seed if in the vicinity of the male fertile. Close mowing stimulates production of short shoots, bearing inflorescences, resulting in the development of large numbers of seeds. Flowering continues throughout the spring, summer and fall.

Chemical control.

Results of the field studies are summarized in Table 1. Only the eight herbicides which gave satisfactory control will be discussed. These were representatives of three major groups: fumigants, soil sterilants and translocated foliage applied herbicides.

Fumigants.

Methyl bromide was the quickest acting and most effective material used. A single application of 1 lb/100 sq ft applied under a plastic tarp was sufficient to completely eradicate kikuyugrass. Appli-

Table 1. Field tests of herbicides on kikuyugrass.

Herbicide	Rate lb/A	Number appl.	Initial control pct.	Regrowth 3-mo pct.	Seedling growth 3 mo
SMDC	450	1	99	2	numerous
Dalapon	8	1	78	39	numerous
Dalapon	8	3	100	none	none
MH	70	1	68	51	numerous
MH	70	2	45	53	numerous
Amitrole	16	1	53	87	numerous
Amitrole	16	2	66	53	numerous
Erbon	675	1	100	none	none
Methyl bromide	435	1	100	none	none
Simazine	8	1	59	73	few
Simazine	8	2	67	59	few
Sodium chlorate sodium borate mixture	1300	1	100	none	none
Dalapon-silvex mixture	45	1	93	7	few
Amitrole + simazine	16+2	1	88	11	few
Amitrole + EPTC	16+6	1	91	3	few
Dalapon + amitrole	5+16	1	73	43	few

cations of 2 lb/100 sq ft were used in several tests where growth was exceptionally heavy or soils were very fine textured. Seeds were destroyed as well as vegetative growth. The necessity of using a plastic cover over the area during application limits the use of methyl bromide to moderate sized areas.

SMDC at 450 lb/A killed all top growth in a single application. However, destruction of seeds was incomplete as numerous seedlings appeared within a few weeks of application. Therefore, successful use of SMDC may be limited to areas containing only the sterile strain. Repeated cultivation or applications of herbicidal oils subsequent to treatment successfully eliminated seedlings but required several months.

Soil sterilants.

Two soil sterilants, erbon and the sodium chlorate–sodium metaborate mixture, completely destroyed all vegetative growth at rates of 657 and 1300 lb/A respectfully. No seedlings were found in treated areas during the 3-month observation period. However, vegetation could not be re-established for a year or longer.

Translocated foliage herbicides.

Dalapon at 8 lb/A was the only herbicide in this group which gave satisfactory control by iself. However, seeds were not destroyed nor was 100 percent control achieved by a single application. Three applications at 4-week intervals gave complete control and prevented new seedling development. This herbicide has proven to be the best material for spot treatment of small patches.

The dalapon-silvex mixture at 45 lb/A gave excellent control, superior to dalapon alone. A single application destroyed nearly all vegetative growth and partially controlled new seedlings.

Amitrole at 16 lb/A followed by simazine at 2 lb/A and amitrole at 16 lb/A followed by EPTC at 6 lb/A gave satisfactory control of vegetative and seedling growth in single applications.

Using two of the effective herbicides above, a kikuyu control technique for home lawns was developed as follows: the entire lawn was first treated with SMDC to destroy vegetative growth. After allowing two weeks for SMDC to dissipate from the soil the ground was cultivated, a seed bed prepared and the lawn seeded to dichondra, a dalapon-tolerant turf-forming ground cover. The contrasting foliage of dichondra made any kikuyu seedlings or new growth from surviving plants readily observable. These were sprayed to wet the foliage with a dalapon solution of 2 oz in 5 gal of water without injury to the dichondra. In this way a lawn was developed and maintained during the long period of kikuyu eradication. When no further kikuyu appears, the lawn may be coverted to a grass if desired.

Chemical control of flowering.

Four chemicals were found to restrict flowering effectively as shown in Table 2. All concentrations of MH, NAA, and 2,4–DEP signifi-

Table 2. Inhibition of flowering of kikuyugrass by various chemicals.

Chemical	Concentration ppm	Total flowers per plant
MH	500	15 *
	1000	7 *
	2000	1 *
NAA	20	67 *
	200	14 *
	2000	3 *
AMO-1618	1000	140 n.s.
	2000	166 n.s.
	3000	207 n.s.
2,4–DEP	5000	107 *
	10000	35 *
	20000	10 *
2,4,5–T	25	141 n.s.
	250	18 *
	2500	0 *
Control—no chemical		176

*Significant at 1% level.
n.s. Not significant.

cantly inhibited flowering as did the higher concentrations of 2,-4,5–T. Injury to the leaves resulted from the highest concentration of MH, 2,4–DEP, and 2,4,5–T. MH at 1000 ppm and NAA at 2000 ppm produced almost complete inhibition of flowering without injury to the foliage. Amo–1618 was not effective at any concentration.

The inhibition of flowering by chemicals might be used to prevent spread of this grass from established erosion control or turf plantings by seed. Small patches of kikuyu are often found within a half-mile radius of established stands of the fertile strain, presumably from seed.

Because of the greater danger of spread by seed, all future kikuyu plantings for erosion control should be of the male sterile strain only.

SUMMARY

Kikuyugrass, *Pennisetum clandestinum*, is a vigorous sub-tropical grass, becoming a serious weed in some parts of the United States. Both a male sterile and a male fertile strain are present in this country.

Chemical control studies showed the following materials to be effective; methyl bromide, SMDC, erbon, sodium chlorate-sodium metaborate mixture, dalapon, dalapon-silvex mixture, amitrole followed by silvex and amitrole followed by EPTC.

A control method for lawns was developed using SMDC fumigation prior to planting dichondra. New kikuyu growth was treated with dalapon as it appeared. Dichondra was not injured by the dalapon spot treatment.

Inhibition of flowering of the fertile strain was accomplished by weekly applications of MH, NAA and 2,4,5–T with no serious injury to vegetative growth.

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Relation of Light Quality to Effects of 2,4–D on Chlorophyll and CO_2 Exchange[1]

GEORGE WILLIAMS and STUART DUNN[2]

MANY of the pathways by which weed-killers, such as 2,4–dichlorophenoxyacetic acid (2,4–D), affect plants are still obscure. Frequently the adverse effect seems to involve an alteration of metabolism, but the exact nature of this is sometimes not clear. Photosynthesis and respiration under different conditions may be either stimulated or inhibited (3, 4, 5, 6). Other results (6), in which a decolorization of tissue with increasing amounts of 2,4–D was observed, suggest that the herbicide either interferes with the formation of chlorophyll, or causes increased destruction.

Previous results of Datta and Dunn (2) with plants grown under colored fluorescent lights showed that 2,4–D reduced dry weight yields, in amounts varying with the main spectral emission of the lamps, as compared to untreated control plants under the same conditions. The pattern of these responses to light quality suggested a possible interference in photosynthesis by the herbicide.

The work reported here attempts to determine if the reduction in dry weight by the herbicide is due to inhibition of photosynthesis, or to some other factor. It tries to get somewhat closer to the actual or inner mechanism than is shown by dry weight yields. This was done in two ways. One was to measure the 2,4–D effects on CO_2 utilization as modified by various light qualities. The second way was to measure these same effects on chlorophyll content. This in turn would have an indirect effect on CO_2 assimilation. There remained the possibility that the dry weight reduction was chiefly or partly due to stimulation of respiration by the herbicide. This respiratory effect was measured by CO_2 production in the dark. All of these herbicidal effects were evaluated in comparison with the responses of control plants under identical conditions.

MATERIALS AND METHODS

Growth of plants and light treatment.

Methods for growing and treating the mustard plants (*Brassica juncea* cultivar Florida Broadleaf) were described previously (2). The

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This paper is based upon a thesis prepared by the senior author in partial fulfillment of the requirements for the Master of Science degree at the University of New Hampshire.

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plants were grown in plastic containers, one pint size, filled with vermiculite and supplied at intervals with a standard nutrient solution. They were kept in a greenhouse for the early part of their growth. Six days after sowing the seed they were thinned to 20 plants per container. After 17 days in the greenhouse the plants were placed on rotating tables under lights of different colors in an air-conditioned basement room. The lamps were all General Electric 96 inch T–8 slimline fluorescent in warm white and five different colors. The distance of each kind of lamp from the plants was adjusted to give a corrected light intensity of 300 foot candles at plant level. Uniform conditions of temperature and photoperiod were maintained as described before (2).

Herbicide treatment.

After a conditioning period of 20 days under the various lights the plants were about 8 to 10 cm in height and still in the vegetative stage. At this time the treatment with a solution of 1000 ppm sodium salt of 2,4–D was started. This was applied as a fine mist from a hand sprayer, thoroughly covering the foliage. In order to allow time for measurements of CO_2 exchange in light and in darkness, all of the plants were not treated at one time. Instead the containers of plants were treated separately at intervals of time sufficient to allow a post-spray period of twenty-four hours under the lights. At the end of this time measurements of CO_2 exchange were made. Appropriate controls of untreated plants were maintained along with the treated ones and were analyzed by the CO_2 exchange technique. At the end of the pre-spray period a set of plants was harvested for fresh and dry weight determinations, to measure the level of their development at this stage. Without giving tabular data, it may be stated that these results showed very similar plant response to the light conditions as those reported previously (2). Again, at the end of each CO_2 analysis both treated and untreated plants were harvested for fresh and dry weights. A fourth set of plants (half treated and half untreated) was allowed to remain under the lights for a longer post-spray period of 10 days, at the end of which time they were sampled for chlorophyll analyses.

Carbon dioxide analyses.

A Liston-Becker Infrared Analyzer, Model 15A, was used to measure the changes in concentration of CO_2. This was connected by Tygon tubing as a part of a closed gas system to the inlet and outlet openings of a plexiglas plant chamber (Figure 1). This chamber was large enough to hold two containers of plants. For the CO_2 determinations in both light and darkness the plants in the chamber were kept in the same position under the lights that they occupied during pre- and post-spray periods. The atmosphere within the system was circulated by means of a Thiberg air-pump at a rate of two cubic feet per hour. The instrument was calibrated before each run

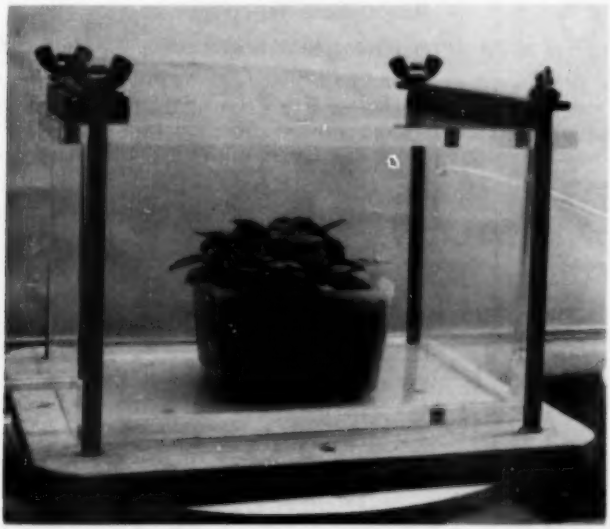

Figure 1. Plexiglas plant chamber used in measuring CO_2 exchanges.

by gas mixtures of known concentration. The results from the amplifier of the instrument were transmitted to an Esterline-Angus recording milliammeter, and could be read from the chart as milliamperes, or as percent of CO_2 in the chamber.

At the beginning of each test, a single container of plants was sealed in the chamber. In preliminary tests it was found that in light the concentration of CO_2 usually levelled off to give a reading near 1.0 or 1.5 milliamperes on the recorder. Therefore, before starting the pump, nitrogen was flushed through the system, bringing the CO_2 concentration down near this level in order to save time. Then the system was closed and the pump started. The lights remained on to allow the plants to reduce the CO_2 concentration still further, to the compensation point. This usually required ten to twenty minutes and then the lights were switched off and the record for CO_2 production in respiration was begun. This took place for one hour, at the end of which time the lights were turned on and CO_2 assimiliation was recorded for another hour. After this the plants were harvested as noted above. This was repeated for a container of treated plants and one of control plants for each of the six positions under all of the different lamps.

Since the lines on the recorder chart for the divisions of time were curved, the points for each ten minutes were read from the chart and replotted on straight-line graph paper. A set of typical curves for warm white light is shown in Figure 2.

RESULTS AND DISCUSSION

The effects of 2,4–D on photosynthesis as modified by light quality.

The downward, or second half of the curves in Figure 2 show that CO_2 was assimilated by the plants in the light, and that the 2,4–D treated plants used it more slowly and to a smaller total extent. This was true in greater or less degree under all light qualities. The

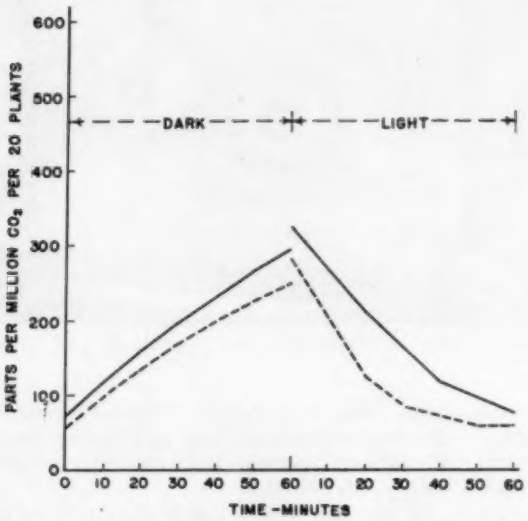

Figure 2. Typical curves showing effects of warm white light on CO_2 exchange by mustard plants, with (——) and without (- - - -) 2,4–D.

area between the broken line curve for the control plants and the solid line curve for the treated plants may be regarded as a measure of the inhibition of apparent photosynthesis by 2,4–D (lag in utilization of CO_2). These areas were all measured by polar planimeter for each pair of curves, and then converted to equivalent microliters of CO_2. The averages of these differences in the volumes of CO_2 used by the plants (2,4–D effect) for six containers of 20 plants each, under each light quality, are shown by the height of the columns in Figure 3(A). The width of each column has no other meaning than to show the extent of the major light emission of each lamp. The significance of results for each set of lamps was calculated by analysis of variance.

These results show that blue light had a marked effect on 2,4–D action as an inhibitor of CO_2 utilization, since the value for this portion of the spectrum was significantly greater at the 1% level than that for any other light quality. There is no significant differ-

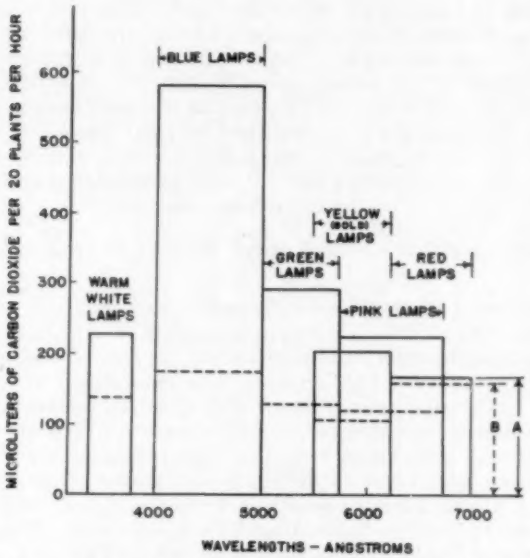

Figure 3. The reduction (A) in light of CO_2 utilization by mustard below control plants, and the promotion (B) in darkness of CO_2 evolution by mustard above control plants by 2,4–D as modified by light quality.
(A)—L.S.D. 233.6(1%), 172.5(5%).
(B)—L.S.D. 75.9(1%), 55.2(5%).

ence between the figures for any of the other lamps, except that the average for red light was significantly less than that for white light at the 5% level. This synergism of blue light and 2,4–D in the inhibition of CO_2 utilization is in agreement with its similar effect with 2,4–D on dry weight yields (2). Also of particular interest is the very low effect of red light on this interference by 2,4–D in CO_2 utilization. Apparently, the reason for the pronounced effect of red light in causing inhibition of growth (dry weight production) by 2,4–D must be sought elsewhere.

The effects of 2,4–D on respiration as modified by light quality.

The upward, or first halves of the curves in Figure 2, are typical of all of those for the different lamps tested, in showing an increase in CO_2 concentration in one hour in the dark. They are also typical in showing that the 2,4–D treated plants evolved CO_2 at a slightly faster rate, in most instances, and in somewhat greater total amounts, than did the untreated controls. Again, the areas between the curves for these two sets of plants and their corresponding amounts of CO_2 in microliters evolved in one hour were taken as being indicative of the 2,4–D effect as modified by light. The averages for all of these

for the different lamps are shown in Figure 3(B). It is evident that none of the differences among these effects of the different portions of the spectrum (plus 2,4–D) on respiration is significant, except that for yellow (gold), which is significantly less than that for blue at the 5% level. However, the pattern of response here is very similar to the effect of 2,4–D as modified by light quality in reducing dry weight yields (2), namely, that it shows a high stimulatory effect on respiration by both blue and red light (combined with the herbicide) and low effects by the light from other lamps.

The effects of 2,4–D on chlorophyll content as modified by light quality.

As mentioned under "Herbicide treatment" certain containers of plants were reserved for chlorophyll analyses. These had been subjected to the same light conditions, herbicide-treated and untreated, as the plants used for CO_2 analysis. The chlorophyll analyses were made on fresh tissue in accordance with standard methods (1), using a Klett-Summerson photoelectric colorimeter, which in turn was calibrated with a Beckman Model DU spectrophotometer. The total chlorophyll content was expressed as milligrams per gram of fresh leaf tissue. Under all light qualities the herbicide caused a decrease in chlorophyll content below that for control plants. The averages of differences between these values, shown in Figure 4, represent the effect of 2,4–D in reducing chlorophyll content.

Here is shown evidence that the reduction in dry weight caused by the combination of red light and 2,4–D is primarily the result of a destruction of chlorophyll or an interference with its formation. The low chlorophyll content would in turn cause low photosynthesis (dry weight yield). This fits in with the fact that red light plus 2,4–D has comparatively little influence on CO_2 utilization in photosynthesis (Figure 3–A). Blue light also has considerable influence on reduction of chlorophyll content by 2,4–D, but not nearly as much as red light. The influence of pink light in this respect is even larger than blue, probably due in large measure to its large proportion of red light (2).

SUMMARY

This report is a continuation of studies on the modifying effects of light quality on herbicide action. Previous results had shown that red light most significantly stimulated the action of 2,4–D in lowering dry weights. Next most effective was blue light. Here an effort was made to determine if this effect on dry weight was due chiefly to interference with CO_2 utilization (apparent photosynthesis) or promotion of CO_2 evolution (respiration). This was done by CO_2 exchange measurements in light and darkness and chlorophyll analyses. The chief results were as follows:

1. Blue light was more influential than all other portions of the spectrum in promoting 2,4–D interference with CO_2 utilization in photosynthesis. Red light was particularly low in this respect.

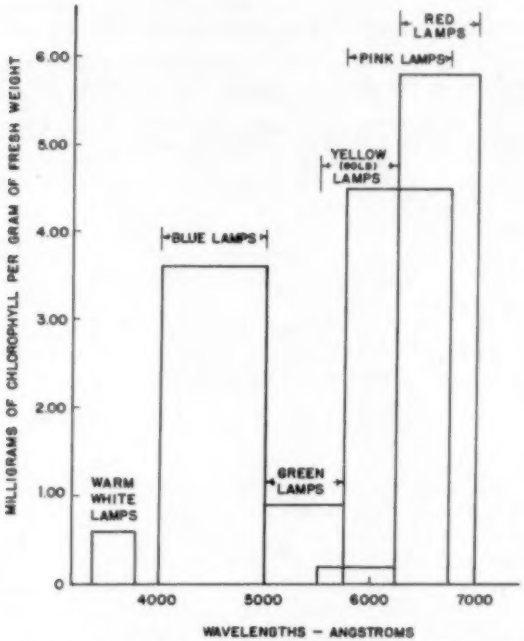

Figure 4. The reduction of chlorophyll content of mustard by 2,4–D below that of control plants as modified by light quality.
L.S.D. 1.44(5%), 2.15(1%).

2. Red light had a large effect in causing low chlorophyll content in combination with 2,4–D. This indicates that the effect of this part of the spectrum in reducing dry weight yield occurs largely through its activity in reducing chlorophyll content, which in turn would lower the photosynthetic efficiency of the plants.

3. The herbicide caused an increase in respiration under all light qualities tested. While the differences between the values for the different spectral regions in this respect were not significant, the pattern of response was very similar to that for dry weight reduction by the herbicide, i.e., red light and blue light in both cases produced the greatest differences between treated and control plants.

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Effects of Light Intensity and Quality on the Growth of *Elodea densa* and *Heteranthera dubia*[1]

R. D. Blackburn, J. M. Lawrence, and D. E. Davis[2]

The need for information concerning effects of quality and quantity of light on submerged aquatic plants has been emphasized by the Committee on Aquatic Weed Control at the Southern Weed Conference (6) and by Westman (9). Factors such as light intensity, water temperature, and depth of water interact with soil fertility to govern the establishment of rooted aquatic vegetation and determine the rate at which it grows (3).

The objectives of this investigation were to determine the effects of selected light intensities and qualities on the growth of *Elodea densa*, (Planch) Caspary (elodea) and *Heteranthera dubia* (Jacquin) Macmillian (water stargrass) under controlled laboratory conditions.

Materials and Methods

Intensity studies.

Preliminary experiments were used to determine light intensity ranges, type of test containers, and other experimental procedures. Seven small isolated growth compartments, 50 × 30 × 36 in., were built in a temperature-controlled laboratory. An individual source of illumination was provided by a two-tube, 48-inch fluorescent fixture attached to the ceiling of each compartment. Each fixture was equipped with two General Electric 40 watt T–12 daylight fluorescent tubes. The period of illumination in each compartment was 12 hours per day. The temperature in each compartment was maintained at approximately 75°F.

Each of the seven compartments contained three aquaria. Aquaria in three of the compartments were stocked with elodea, and aquaria in the remaining four compartments were stocked with water stargrass. Three light intensities were used in each compartment, and were regulated by varying the distance of the aquaria from the light source or by shading the aquaria with cheesecloth. The light intensities used on each species were as follows:

Compartment No.	Plant	Foot-candles on an aquarium	Intensity range
1	Elodea	5, 7, and 10	Low
2	Elodea	40, 68, and 83	Medium
3	Elodea	125, 142, and 170	High
4	Water stargrass	5, 7, and 10	Low
5	Water stargrass	40, 68, and 83	Medium
6	Water stargrass	125, 142, and 170	High
7	Water stargrass	245, 395, and 591	Maximum

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The differences in growth of plants under different intensities within each compartment were too small to be significant and hence they have been lumped together into intensity ranges in presentation and discussion of data.

Light intensities were measured at the water surface with a Model 756, Weston illumination meter equipped with uncorrected photocells. The Weston meter was also used with Kodak A, B, C–5, and K–2 series of VI Wratten filters to determine the spectral composition of the light source. Paired filters of the same color were placed over the glass openings of the meter head and were used to determine relative light intensities present in different portions of the visible spectrum for the light source used. No attempt was made to correct for different sensitivities of the light meter to various wave lengths.

Plants were grown in slate bottom aquaria with glass sides, 24 × 12 × 12 in. Aquaria were filled with dechlorinated tap water to a depth of 9 in., and were refilled at 6 weeks to compensate for evaporation losses. The water had a calcium content of 25 to 29 ppm and a pH of 8.6 to 8.8. A mineral salt solution which contained 12 mg nitrogen, 6 mg phosphoric acid, 6 mg potash, and 2.4 mg calcium carbonate was added to each aquarium at 3 and 45 days after plants were stocked.

Vigorous, growing, stem-tip cuttings of elodea and water stargrass were kept in the laboratory for one week prior to use in tests. At the end of this period, all stem-tip cuttings were trimmed to 100 mm in length. A ¼-in. diameter glass tube ¾ in. long was slipped over the basal or cut end of each cutting to make it sink to the bottom of the aquarium. In the test there were 7 cuttings in each of 3 rows or a total of 21 cuttings per aquarium. Thus, 21 stem-tip cuttings of each species were used under each light intensity. Growth measurements and observations were made at 2-week intervals for the 12-week test period.

Quality studies.

Four isolated growth compartments similar to those used in the intensity studies were used in the light quality studies. Each compartment was provided with the same illumination equipment as described in the intensity study, except that the colors of fluorescent tubes used were black, blue, green, and red rather than white. The wave lengths of light emission by these tubes were 3,200 to 4,500 Å for the black, 3,800 to 5,600 Å for the blue, 4,800 to 6,300 Å for the green, and 5,900 to 7,200 Å for the red light. This range of colored lights covered the entire visible portion of the spectrum.

The light quality tests were performed as described for the intensity studies except for the light source and number of plants. The light intensity used for all elodea tests was 10 foot-candles. Light intensities for the elodea test were maintained at 10 foot-candles by regulating the distance of aquaria from light source and by shading with cheesecloth. The intensities of colored lights used for water stargrass were: green, 225 foot-candles; blue, 145 foot-candles; red, 125 foot-candles; and black, 117 foot-candles. Each

compartment contained two aquaria, of which one contained 24 elodea plants and one contained 24 water stargrass plants, all arranged in three rows of 8 plants each.

Observations of plant conditions and stem-tip growth measurements of each species were recorded at 2-week intervals for the 12-week test period.

RESULTS AND DISCUSSION

Intensity studies using elodea.

Elodea grew throughout the 12-week test period under the low intensity range. In the medium range, the elodea grew at a rate somewhat similar to the low intensity for the first 6 weeks of the test, but thereafter the leaves began to turn yellow and the stems eventually died. Elodea grew in the high intensity range very fast for the first 2 weeks of the test. After the first 2 weeks the growth began decreasing and the plants turned yellow. Plants began to die by the 8th week and nearly all were dead by the 12th week.

The average growth for a compartment for each time increment is presented in Table 1. These averages were derived only from

Table 1. Growth of elodea, expressed as average total length in mm of surviving plants for every 2-week period, under various light intensities.[a]

Growth period Weeks	Average total length in mm of elodea cuttings under various light intensities		
	Low	Medium	High
0	100	100	100
2	138	127	123
4	144	130	124
6	146	131	126
8	147	131	126
10	148	129	124
12	149	121	108

[a]Low light intensities, 5, 7, and 10 foot-candles.
Medium light intensities, 52, 68, and 82 foot-candles.
High light intensities, 125, 142, and 170 foot-candles.

the living plants. Thus, the plants appear to be growing better than they actually were. Out of 63 plants, the numbers surviving the 12-week test period for each light intensity were: low intensity 61, medium intensity 15, high intensity 4.

Ordinarily long time growth increases with increasing light intensity until some other factor becomes limiting. High light intensities have been reported to have an inhibitory effect upon photosynthesis and involve a phenomenon called solarization (5). Short periods of high intensities are usually not harmful to leaves or other photosynthetic organs, but continuation of this high light intensity for a long period results in decolorization of chlorophylls and ultimately in death of the cells. High light intensities on elodea caused a discolorization of the chlorophylls in leaves, cessation of growth, and then death of the plant.

Under the conditions of this experiment, optimum light intensity for elodea growing under white fluorescent lamps was somewhere near 10 foot-candles. It can be concluded that aquatic weed control measures that reduce light intensity will be only partially effective on elodea because of its low light requirements.

Intensity studies using water stargrass.

Maximum growth of water stargrass occurred at the highest light intensity used, indicating that the optimum intensity under white fluorescent lamps is probably at or above 590 foot-candles (Table 2).

Table 2. Growth of water stargrass, expressed as average total length in mm of surviving plants for every 2-week period, under various light intensities.[a]

Growth period	Average total length in mm of water stargrass cuttings under various light intensities			
Weeks	Low	Medium	High	Maximum
0	100	100	100	100
2	109	110	113	135
4	111	115	120	161
6	113	116	123	183
8	115	118	126	209
10	114	119	128	220
12	108	120	132	232

[a]Low light intensities, 5, 7, and 10 foot-candles.
Medium light intensities, 40, 68, and 83 foot-candles.
High light intensities, 125, 142, and 170 foot-candles.
Maximum light intensities, 245, 395, and 591 foot-candles.

Most of the plants grown under the lowest range of intensities did not survive the 12-week test period. The leaves of the plants began to turn yellow, and stems became spindly after the 6th week. Survival of the original 63 plants for the 12-week test for each intensity range is as follows: low intensity 7, medium intensity 61, high intensity 63, and maximum intensity 63.

It can be assumed that the low light intensity range was too low for sufficient photosynthesis to compensate for both day and night respiration. Growth of plants can be maintained only if a supply of food is available for the growing parts (4). As the light intensity was increased from 40 to 590 foot-candles, the growth of the water stargrass also increased.

Shading is a possible control measure for water stargrass because of its high light requirement.

Quality studies using elodea and water stargrass.

There are many factors that must be considered before these intensities, measured with a Weston meter with an uncorrected glass photocell, can be correlated with photosynthetic efficiencies of the various plants. First, the Weston cell is not equally sensitive to all wave lengths. Second, water does not transmit all wave lengths equally well. Hence the water not only reduced the light intensity but also changed its quality. Third, not all wave lengths of light

Table 3. Growth of elodea, expressed as average total length in mm of surviving plants for every 2-week period, under various light sources.[a]

Growth period Weeks	Average total length in mm of elodea cuttings under various light sources			
	Black	Blue	Green	Red
0	100	100	100	100
2	108	121	105	125
4	108	121	105	128
6	114	130	106	132
8	123	135	107	136
10	110	148	107	139
12	0	147	110	149

[a]Light intensity on water surface for all sources was 10 foot-candles.

are equally effective in inducing photosynthesis in the intact plant.

There was no growth of water stargrass after 4 weeks under black lamp illumination (Table 3) or of elodea after eight weeks (Table 4). All plants under this light source were dead at the end of 12 weeks, and the results were very similar to those obtained when the two species were grown in complete darkness. It was assumed that death under this lamp resulted from lack of the proper wave lengths of light for photosynthesis rather than any destructive action of the light itself.

Under the blue lamps, growth of water stargrass ceased at 8 weeks with only 5 of the 24 plants surviving the 12-week test period. Elodea ceased growth under this lamp at 6 weeks with only 2 of the 24 plants surviving for 12 weeks. This was somewhat surprising, since blue light penetrates water well and since Went (8) has shown that certain seed plants have their second maximum photosynthesis at 4,400 Å or the blue portion of the spectrum. However, other physiological processes than photosynthesis may be involved.

Growth of water stargrass under the green lamp was approximately three times greater during the second 6 weeks of testing than that obtained under the daylight lamps of similar intensity. Elodea growing under the green lamp began turning yellow the

Table 4. Growth of water stargrass, expressed as average total length in mm of surviving plants for every 2-week period, under various light sources.

Growth period Weeks	Average total length in mm of water stargrass cuttings under various light sources[a]			
	Black	Blue	Green	Red
0	100	100	100	100
2	102	111	116	123
4	103	117	116	158
6	103	123	118	183
8	0	126	135	191
10	0	124	151	196
12	0	120	166	219

[a]Light intensity, black 117 foot-candles.
Light intensity, blue 145 foot-candles.
Light intensity, green 225 foot-candles.
Light intensity, red 125 foot-candles.

2nd week and began dying the 6th week, with only three plants surviving the 12 weeks. Solarization of chlorophyll appeared to be the cause of yellowing and eventual death of the elodea plants. Gabrielsen (1) found that wheat had its second highest rate of photosynthesis in the green light, while *Ulva lactuca,* a marine alga, had its second highest rate of photosynthesis in the blue light.

It has been shown by many investigators that the highest rate of photosynthesis in seed plants and algae occurs in orange-red light (1, 2, 8). It would be expected that the water would absorb some of the red or long wave lengths of light. Nevertheless, both elodea and water stargrass made their best growth under the red lamp.

SUMMARY

1. Techniques for growing elodea and water stargrass in a light and temperature controlled laboratory were developed.

2. The optimum intensity for *Elodea densa* under daylight fluorescent lamps was somewhere near 10 foot-candles. Light intensities above 125 foot-candles caused a decolorization or solarization effect on the chlorophyll of elodea in 2 weeks.

3. The optimum intensity for *Heteranthera dubia* was at or above 590 foot-candles. There was a corresponding decrease in growth as the light intensity decreased below 590 foot-candles.

4. Both elodea and water stargrass failed to survive for the 12-week test period under the black lamp. A majority of plants of both elodea and water stargrass did not survive the 12-week period under the blue lamp.

5. Elodea growing under green light began to turn yellow and show effects of solarization at the end of 2 weeks. Water stargrass grew almost 3 times as fast under green as under daylight lamps of similar intensity. Maximum growth of water stargrass occurred during the 6th to 12th week of tests.

6. Both species grew very rapidly when exposed to the red lamp.

7. Control by shading, i.e. by fertilization or by dyes, may be a possible control measure for water stargrass, since it has a high light requirement. However, this method would not appear suitable for control of elodea, since this species has a low light requirement.

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Influence of Nozzle Size and Type Hardness of Water, and Wetting Agents on Spray Drift[1]

Glen E. Page[2]

Introduction

Drift of spray droplets is a major problem in the application of insecticides and herbicides. Spray drift is the movement of the spray from the place of application to adjacent areas by wind or air currents. Many insecticides and herbicides are effective in minute quantities and even the small amount that drifts can be harmful to crops and livestock. Parathion, 2,4–D, and 2,4,5–T are among those chemicals whose drift is a problem where they are used.

It is impossible to find a time when there is no air movement in the field. The forward motion of a ground sprayer will cause some air movement. It has been found by Smith (6) that no nozzle produces droplets of a uniform size, but rather, produces a continuous range of droplet sizes. Akesson, Harvey (1) and Frensh (4) showed that with a given nozzle, the range of droplet sizes narrowed, and the number of small droplets increase as pressure increases. As orifice size and nozzle capacity increase, the average droplet size also increases, but the small droplets typical of small orifice delivery are not eliminated. This paper is concerned with these small droplets which drift great distances with a small amount of air movement and the work done at Oregon State College relating to some of the factors influencing drift.

Equipment and Procedure

A wind tunnel 29 inches square and 39 feet overall length was used in conducting the tests, (Figures 1 and 2). A double-width, double-inlet, forward curved fan with a 21 inch wheel discharged into a short plenum chamber. The air velocity in the tunnel was controlled by an air metering valve (Figure 3) located between the plenum chamber and the spray chamber of the wind tunnel. The valve consisted of two sheets of ¼ inch plywood match drilled with 217 one-inch holes. By sliding one drilled sheet in relation to the other, the size of the openings were varied to control the air flow. The air was then forced through a two-inch thick furnace type fiberglass air filter. The air valve and filter act as a static pressure screen to insure uniform air flow in the tunnel. Air velocities in the tunnel were determined with a thermal anemometer. Anemometer readings were taken on a grid pattern across the tunnel to obtain average velocities. All tests were run with the air valve set to give an air velocity of 88 feet per minute (1 MPH). The first section of the tunnel was used as a spray chamber. Windows in both sides

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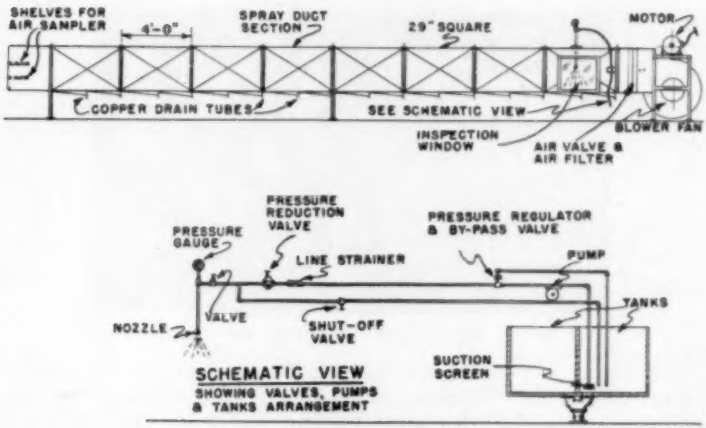

Figure 1. Spray drift tunnel layout.

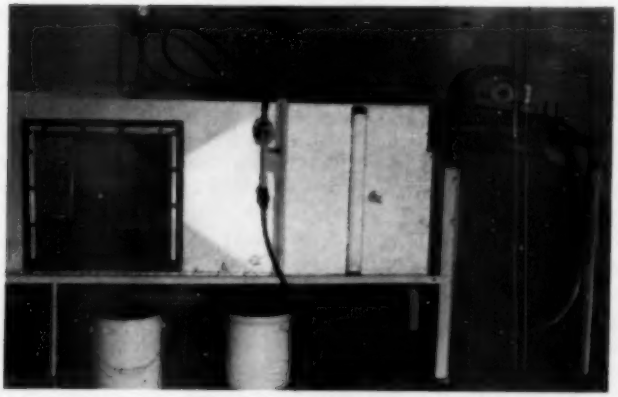

Figure 2. Fan, plenum, and spray chamber section of spray tunnel.

of this section permitted observation of the spray in this chamber. All drift samples were collected at a point 32 feet from the nozzle by two high volume air samplers located just inside the discharge end of the tunnel. Approximately 18 cubic feet of air per minute was drawn through the filters by the air samplers. A roller pump was used for spraying and providing hydraulic agitation in the spray tank. The pump was protected by a pressure relief valve with bypass set 4 to 5 psi above desired spraying pressure. A diaphram pressure reducing vale regulated the spraying pressure at the nozzle.

A standard spray solution containing two grams of a water soluble dye per gallon of water was used. To this standard solution, wetting

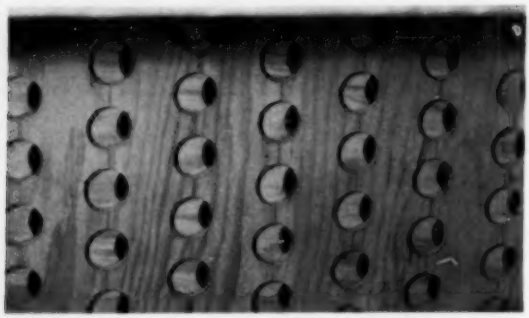

Figure 3. A partial view of the air metering valve.

agents and other chemicals to be tested were added. The spray solution was agitated for several minutes to insure thorough mixing before starting a test. The fan and air samplers were started before turning on the spray nozzle for a precise length of time, usually 20 minutes. While the test was in process, two samples of the spray solution were taken from the spray tank for color intensity determination.

The dye collected on the filters was removed by soaking in 25 c.c. of distilled water for 10 minutes and agitating periodically during the washing period. Color intensity of the resulting wash solution and of the sample of spray solution was determined with a colorimeter. In setting up this procedure it was found that increasing the washing period beyond 10 minutes resulted in no change in colorimeter readings.

The amount of drift was determined by comparing the color intensity reading with a calibration chart.

SCOPE OF TESTS

Tests were run to determine the effect of nozzle size, nozzle spray angle, and nozzle type, and the interaction in relation to spray drift. Three nozzle sizes, 0.1, 0.2, and 0.3 gallons per minute at 40 psi gauge pressure; two spray angles, 65° and 80°; and two nozzle types, the flat fan and the hollow cone, were tested (Table 1). Tests were run at air speed of 1 mile per hour and replicated six times.

Tests were run to determine the effect of different wetting agents and the U.S. degree of water hardness (3) on spray drift. Three wetting agents (Table 2) Vatsol OT, sodium dioctyl sulfo-succinate, ionic; Triton 114, water soluble octyl phenoxy polyethoxy ethanol, non ionic; X77, alkylargyl polyoxyethylene glycol in isopropyl alcohol, non ionic; were used at concentrations by weight of 0.1 and 0.01 percent. Waters with hardness ratings 2.5 and 25 degrees were used. These tests were run with a 0.2 gpm 80° flat fan nozzle at 40 psi gauge. Tests were replicated three times.

Table 1. Nozzle treatments used showing calibrated rating spray angle and nozzle type.

Nozzle No.	Calib. gal/min	Spray angle	Type
111	0.10	65°	Fan
211	0.20	65°	Fan
311	0.28	65°	Fan
121	0.08	67°	Hollow cone
221	0.17	61°	Hollow cone
321	0.34	69°	Hollow cone
112	0.10	80°	Fan
212	0.20	80°	Fan
312	0.29	80°	Fan
122	0.12	79°	Hollow cone
222	0.18	89°	Hollow cone
322	0.38	79°	Hollow cone

Table 2. Concentrations of wetting agents used and water hardness ratings before addition of wetting agents.

Treatment solution No.	Wetting agent	Type agent	Concentration pct. by wt.	U.S. degree hardness
1	Vatsol OT	ionic	0.01	25.0
2	Vatsol OT	ionic	0.01	2.5
3	X77	non-ionic	0.01	25.0
4	X77	non-ionic	0.01	2.5
5	Triton 114	non-ionic	0.01	25.0
6	Triton 114	non-ionic	0.01	2.5
7	Vatsol OT	ionic	0.1	25.0
8	Vatsol OT	ionic	0.1	2.5
9	X77	non-ionic	0.1	25.0
10	X77	non-ionic	0.1	2.5
11	Triton 114	non-ionic	0.1	25.0
12	Triton 114	non-ionic	0.1	2.5
13	None		None	None

RESULTS

Spray drift data were expressed in terms of gallons drift per 100 gallons of solution sprayed. The data on the effect of nozzles on drift were analyzed statistically and summarized in Tables 3, 4 and 5. The variables of nozzle type, size and spray angle resulted in highly significant differences in the amounts of spray drift measured. Spray drift decreased with increase in nozzle size, and increased with increase of spray angle. The interaction of nozzle type by angle was

Table 3. Effect of nozzle size and type on spray drift, expressed in gallons drift per 100 gallons sprayed.

Nozzle type	Nozzle size gpm			
	0.08–0.12	0.17–0.20	0.28–0.38	Type means
Fan	0.717	0.461	0.367	.515
Hollow cone	0.619	0.452	0.256	.432
Size means	.668	.442	.311	

LSD at 1% level: Size, 0.045; Type, 0.036; Size × type, 0.063.

Table 4. Effect of nozzle and spray angle on spray drift, expressed in gallons drift per 100 gallons sprayed.

Spray angle	Nozzle size, gpm			
	0.08–0.12	0.17–0.20	0.28–0.38	Angle means
61–69°	0.548	0.428	0.287	0.421
79–89°	0.787	0.455	0.336	0.526
Size means	0.668	0.442	0.311	

LSD at 1% level: Size, 0.045; Angle, 0.036; Size × angle; 0.063.

Table 5. Effect of nozzle type and spray angle on spray drift expressed in gallons per 100 gallons sprayed.

Spray angle	Type		
	Fan	Hollow cone	Angle means
61–69°	0.444	0.399	0.421
79–89°	0.586	0.466	0.526
Type means	0.515	0.432	

LSD at 1% level, type × angle 0.052.

Table 6. Effect of wetting agent and concentration of wetting agent on spray drift, gallons per 100 gallons sprayed.

Conc. pct. by wt.	Vatsol OT	X77	114	Conc. mean
0.01	.294	.328	0.305	0.309
0.1	.332	.339	0.437	0.369
Agent means	.313	.336	0.369	

LSD	5%	1%
Agent × concentration	.056	.075
Concentration	.030	.044
Agent	.038	.053

highly significant on amount of spray drift measured, the interaction of type by size was not significant.

The data on the effect of water hardness and wetting agents on drift were analyzed statistically and summarized in Table 6. The degree of water hardness had no significant effect on the amount of drift measured. Surface tension is an important factor in droplet size and drift, but under the test conditions, the only wetting agent that had a significant effect on the amount of drift was Triton 114 at a concentration of 0.1 percent by weight.

To further check the effect of Triton 114, tests were run at concentrations of 0.00, 0.01%, 0.05% and 0.1% of Triton 114 in soft water. Each test was replicated three times. Only at a concentration of 0.1 percent of Triton 114 was there a significant difference in drift.

SUMMARY

1. Tests were conducted in a wind tunnel at low levels of air movement to determine the effect on spray drift of nozzle type,

size, and spray angle. Tests were conducted to determine the effect on spray drift of wetting agents Vatsol OT, X77, and Triton 114 at percent concentrations by weight of 0.01 and 0.1.

2. The spray drift decreased with increased nozzle size.

3. Spray drift was greater for nozzles with 80° spray angle than for 65° spray angle at discharge rate of 0.1 gallon per minute.

4. It was impossible to get a clear-cut comparison of the effect of fan nozzles and of hollow cone nozzles on spray drift.

5. Triton 114 at a concentration of 0.1 percent in spray solution increased spray drift significantly.

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Some Physiological Effects of 2,2–Dichloropropionic Acid[1]

M. Ingle[2] and B. J. Rogers

The sodium salt of 2,2–dichloropropionic acid (dalapon) has found a place in chemical weed control practices because of its effectiveness against grasses. The nature of its action is understood only poorly.

Fawcett et al. (5) found that dalapon produced a slight increase (significant at the 1% level) in wheat coleoptile elongation at slightly less than 10^{-3}M, but no inhibition over the range of 10^{-2}M to 10^{-6}M. Other chlorinated acetic acid derivatives promoted greater elongation. All of the compounds in this class which were tested proved to be inactive in the pea curvature test.

Recognizing structural similarities, Redemann and Meikle (15) investigated the effect of dalapon on bacterial pyruvate oxidase and yeast pyruvate decarboxylase. Lineweaver-Burk plots indicated simultaneous competitive and uncompetitive inhibition. The latter inhibition was thought to result from protein precipitation; later studies showed that a much higher concentration of dalapon was required to precipitate a significant amount of protein (16).

Compared with other herbicides, dalapon has relatively little effect on oxygen uptake. Switzer (20) reported that dalapon at 5×10^{-2}M was required for 50% inhibition of oxygen uptake by soybean mitochondria. Approximately the same concentration of dalapon was required for inhibition of oxygen uptake by three species of *Azotobacter*, while the gaseous exchange of soil enriched with nitrifying bacteria was little affected by dalapon (9, 14).

Hilton et al. (10, 11) have shown that dalapon and other chlorinated aliphatic acids interfere with pantothenic acid metabolism in microorganisms. The first work, on the growth of yeast, indicated that there was a competitive relationship between 2,3,3–trichloroisobutyric acid or 2,3–dichloroisobutyric acid and beta-alanine. The chlorinated compounds were more inhibitory than the unsubstituted forms. Beta-alanine provided some protection against dalapon inhibition, but the protective effect was not as great as when the isobutyric acid derivatives were used as inhibitors, nor was the competitive effect between beta-alanine and dalapon as clearly demonstrable.

Subsequent studies with the pantothenate-synthesizing enzyme from *Escherichia coli* have shown that there is a competitive relationship between pantoic acid and dalapon, and an uncompetitive relationship between beta-alanine and dalapon (11). The same phenomena could not be demonstrated readily in higher plants, although there was some evidence of antagonism between pantothenic acid and dalapon. Hilton concluded on the basis of his work

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that the total herbicidal action of dalapon cannot be ascribed to interference with pantothenate synthesis, but that the pantothenate system is perhaps one of the metabolic sites sensitive to the herbicide.

In the present investigation, an attempt has been made to study some physiological responses of plants to dalapon. The results presented here should be taken as a contribution to a larger body of experimental facts which must be prerequisite to an understanding of any "herbicidal mode of action".

METHODS

In our investigation the effect of dalapon on root elongation of two species, corn and cucumber, was studied. The corn root growth test was an adaptation of a method devised by Swanson (19). Corn seed (WF9 × M14) was surface-sterilized by soaking for 1 minute in 5.25% sodium hypochlorite solution (Purex) and rinsed 3 times with sterile de-ionized water. The seeds were placed in sterile enamel trays lined with 2 layers of Whatman #1 filter paper wetted with de-ionized water. The trays were covered with aluminum foil and placed in an incubator at 32°C. After 36 hours, when the primary roots were 20–30 mm in length, 10 intact seedlings were transferred to sterile Petri dishes (150 mm × 20 mm) which contained 2 discs of filter paper and 15 ml of the treatment solution. The seedlings were chosen so that the lengths of the roots fell within a 7 mm range, with a maximum length of 32 mm. All operations were carried out in a culture room where the atmospheric content of microbial spores was low. The lengths of the primary roots were determined at the time of transfer to the treatment dishes and again after 24 hours at 32°C. Each point in the data represents an average of the growth of 50 roots.

Approximately 35 cucumber seeds (variety Marketer) were placed in Petri dishes (100 mm × 10 mm) which contained 2 discs of Whatman #2 filter paper and 3 ml of de-ionized water. After 36–42 hours at 27°C in the dark, the primary roots were measured and transferred to Petri dishes which contained 2 discs of filter paper and 3 ml of the test solution. A 24-hour growth period was used. The initial root lengths were within a 5 mm range, with 19 mm the maximum length. Each point in the data represents an average of the growth of 70 roots.

All treatment solutions were buffered with either 0.01 M phosphate or 0.01 M citrate buffer. Preliminary experiments showed that these buffers at the concentrations indicated had no effect on root growth, and the response of the roots to dalapon was not modified by either of the buffers.

Mitochondria from corn roots were prepared by the method of Lund et al. (13) and from castor bean endosperm by the method of Beevers and Walker (2). Oxygen and phosphate uptake were determined over a 30 minute period. Oxygen uptake was measured manometrically. Initial and final phosphate concentrations were

measured by the method of Allen (1). Each experiment was replicated 4 times.

RESULTS AND DISCUSSION

At pH 4.0, corn root growth was inhibited at concentrations of dalapon greater than 10^{-3}M, while cucumber root growth, in the same time interval and at the same pH, was inhibited by concentrations of greater than 5×10^{-4}M (Figure 1). The concentration required for 50% inhibition of elongation at pH 4.0 was 5×10^{-3}M for corn roots, and 1.05×10^{-3}M for cucumber roots. At 10^{-2}M the degree of inhibition was much the same for both species.

Since there is evidence that the biological activity of acidic organic compounds increases with increasing hydrogen-ion concentration toward the pK_a (18), we suspected that the activity of dalapon would be influenced by the pH of the treatment solution. Figure 2 shows

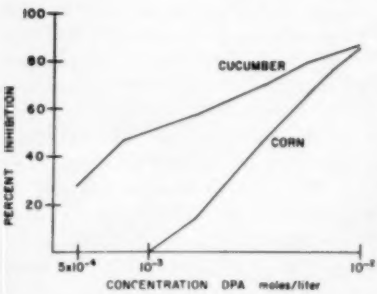

Figure 1. (Left) Comparative effect of dalapon (DPA) on growth of corn and cucumber primary roots.

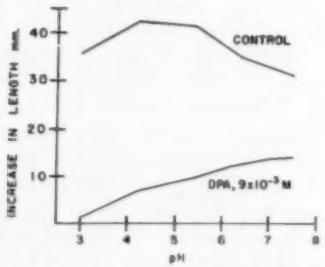

Figure 2. (Right) The effect of pH on the growth of corn primary roots in the presence and absence of dalapon (DPA).

the growth of corn roots, with and without dalapon, over the range pH 3.0 to 7.4. Growth of the controls was equal at pH 4.2 and 5.2, and fell off at the extreme pH values. There was a regular decrease in root elongation in response to dalapon as the hydrogen-ion concentration increased, as reported by Foy (6).

The pK_a of dalapon was estimated from a titration curve to be approximately 1.6. Using this value, the concentration of undissociated molecules was calculated and plotted against percent of control growth. The evidence (Figure 3) indicates that there is a linear relationship between the concentration of undissociated dalapon molecules and growth response of corn roots within the range pH 3.0 to 7.4.

The response of cucumber roots to several hydrogen-ion concentrations at one dalapon concentration was not determined in a single experiment; however, concentration series were run at several pH levels. As with corn roots, inhibition of growth increased

with increasing hydrogen-ion concentration in the presence of dalapon. It was noticed that as the dalapon concentration increased, pH had progressively less effect. At a dalapon concentration of 5×10^{-4}M there was no inhibition of root elongation at pH 7.0, and 40% at pH 4.0. At 10^{-2}M, inhibition at pH 7.0 was 70% and at pH 4.0, 80%.

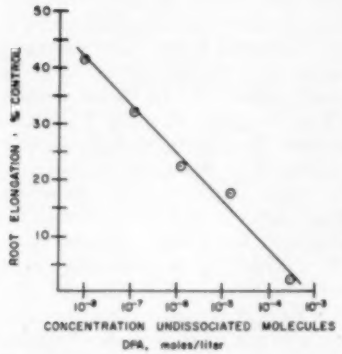

Figure 3. (Left) Effect of the concentration of undissociated molecules of dalapon (DPA) on growth of corn primary roots. Differences in concentration produced by varying the pH.

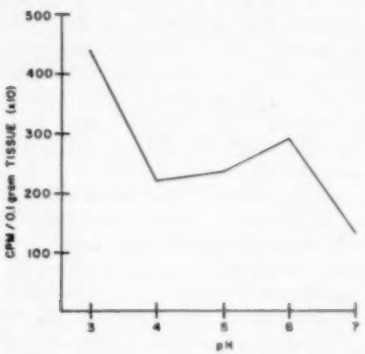

Figure 4. (Right) Uptake of 2-C^{14}-dalapon by corn primary roots as a function of pH.

To determine if increased toxicity at higher hydrogen-ion concentrations was the result of greater dalapon uptake, an experiment utilizing dalapon–2–C^{14} [3] was performed. Corn was germinated as previously described and after 40 hours transferred to Petri dishes (100 mm × 10 mm) which contained 3.5 ml Sorensen's citrate-phosphate buffer, diluted 1:10, and 0.5 μc dalapon–C^{14} (spec. act. 0.948 mc/mM) as the sodium salt. After 4 hours incubation at 27°C, the primary roots were excised, rinsed in running water for 30 seconds, blotted dry, and placed in boiling 80% ethanol for 5 minutes. The material was homogenized in the ethanol and filtered; the filtrate pH was adjusted to 7.0 and the volume reduced to 10 ml by boiling. One ml aliquots were withdrawn, placed in stainless steel counting cups, dried, and the activity counted with a G–M tube to a level of 2% accuracy. The experiment was replicated 3 times.

The data (Figure 4) suggest that uptake is promoted by increasing the hydrogen-ion concentration. Uptake was more or less constant from pH 4.0 to pH 6.0, but rose sharply at pH 3.0 and fell at pH 7.0. This does not correspond fully with the growth data, where there was a consistent relationship between pH and root elongation. Lack of a linear correlation between uptake and growth response

[3]Supplied by the Dow Chemical Co.

indicates that factors other than the amount of inhibitor in the tissue may be involved. As emphasized by Simon and Beevers (18) the relative activity of undissociated molecules and ions cannot be properly evaluated until the pH at the site of action is known.

The time necessary for inhibition of root growth by dalapon was studied. Root growth of corn and cucumber seedlings was measured during a 24 hour exposure to a concentration of 5×10^{-3}M dalapon at pH 4.0. For both species inhibition was apparent after the first 4 hours, and after 8 hours little further increase in length occurred. In another study, corn seedlings were removed from the treatment dishes after various periods of exposure to 5×10^{-3}M dalapon at pH 4.0. The seedlings were rinsed and transferred to dishes which contained buffer at the same pH. The lengths of roots 24 hours after the original transfer to dalapon were compared. A slight inhibition (10%) occurred after 1 hour exposure to dalapon; at 8 hours inhibition was 75%. Inhibition was nearly linear with exposures up to 8 hours; longer exposures caused no further inhibition.

It seemed logical that inhibition of this magnitude would have some effect on oxygen uptake, or be caused by interference with respiration. Therefore, the oxygen uptake of excised corn root tips was measured manometrically. After a period for equilibration and establishment of a basal rate, dalapon was added to one set of flasks. Oxygen uptake was followed for 6 hours, an interval in which significant growth inhibition occurred, as shown in the preceding experiments. No change in oxygen uptake due to dalapon was observed (Figure 5). Similar results were obtained with cucumber root sections.

When slices of carrot root or other storage tissues are incubated with water or dilute buffer the rate of oxygen uptake increases with time from cutting (12, 21). Treatment of fresh tissue with 2,4–dinitrophenol (DNP) causes a very large and rapid respiratory increment, but the rate does not change appreciably over a long period. The increments due to aging and DNP appear to differ (12); the effect of dalapon was compared with these two increment types. Small sections of carrot root (8 mm dia., 1 mm thick) were placed in Warburg vessels which contained solutions of either 2.5×10^{-5}M DNP or dalapon at 7.5×10^{-3}M and 5.0×10^{-3}M, all buffered at pH 5.6 with M/30 citrate. For 8 hours there was a fairly steady increase up to 166% of the initial rate in oxygen uptake by the slices incubated in buffer. The DNP caused a 214% increase in oxygen uptake during the first hour; this rate fell off during the subsequent 7 hours to about 70% of the initial rate. Dalapon at 5×10^{-3}M slightly inhibited the increment due to aging; at 7.5×10^{-3}M this increment was prevented. These results suggest that the metabolic action of dalapon and DNP are quite different in nature.

The effects of dalapon on some endergonic process dependent on metabolic energy derived from aerobic respiration was determined. The uptake of phosphate ion was chosen as such a system. The method used was patterned after that of Hagen and Hopkins (8).

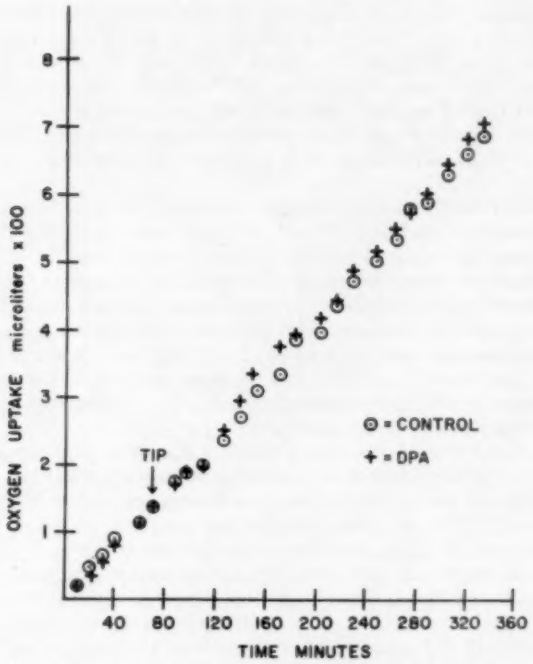

Figure 5. Oxygen uptake by corn root tips. Dalapon (DPA) added at "TIP".

Corn seedlings were pretreated with either 10^{-4}M DNP or 5×10^{-3} M dalapon in 0.01M citrate buffer, pH 4.0. Both treatments produced equivalent growth responses. After 12 hours the primary roots were excised, rinsed, and aliquots placed in absorption flasks which contained 1000 ml of 8.4×10^{-5}M KH_2PO_4 solution with a specific activity of 4.0 mc/gram of phosphate. The flasks were aerated constantly. After 3 hours the roots were removed, rinsed, placed in planchets, dried, and then counted with a G–M tube. The experiment was replicated 3 times. Uptake of radioactive phosphate was found to be linear, which is in accord with Butler's report (4). The 12 hour pretreatment with dalapon reduced the uptake of P^{32} by 50%, while the DNP caused a 25% reduction. Data from one experiment gave these CPM per gram of dry tissue: buffer pretreatment, 6300; DNP 5000; dalapon, 3200.

The possibility that dalapon might interfere with oxidative phosphorylation was explored. Mitochondria were prepared from corn roots and from castor bean endosperm. Neither dalapon nor DNP had a significant effect on oxygen uptake. Orthophosphate disappearance indicated a small but consistent inhibition of phos-

phate uptake caused by dalapon in both preparations. DNP was more effective in causing a depression in phosphate uptake, by a factor of 30 to 60%. When hexokinase and fluoride were not included in the reaction mixture, DNP almost doubled the oxygen uptake in the 30 minutes, but dalapon had no effect. We concluded that, while dalapon may have some effect on oxidative phosphorylation, it is relatively slight and does not account for the total inhibition.

Certain growth regulators do produce changes in respiration. Indole–3–acetic acid and 2,4–D enhance oat coleoptile elongation, with a simultaneous increase in oxygen consumption (3, 7). At higher concentrations these compounds inhibit both growth and respiration. DNP and 2,4–dichlorophenol cause an increase in oxygen uptake at concentrations which cause almost complete growth inhibition of coleoptiles and corn roots (3, 7). Apparently there is another class of compounds which may inhibit growth without affecting respiration significantly. Canavanine is one of these compounds (3), and dalapon seems to be another.

The effects of dalapon on growth, phosphate uptake by excised roots, and the respiration of carrot slices suggest that dalapon interferes with the utilization of metabolic energy rather than with its production. That is, specific reactions involved in growth or some other process utilizing metabolic energy are inhibited. The inhibition by dalapon of the respiratory increment brought about in carrot tissue by aging is an example. There is reason to believe that this increment may be partially due to increased endergonic processes (12). If dalapon were to interfere with some such process, the basal respiration of the tissue would be unaffected.

Following Hilton et al. (10, 11), we attempted to reverse dalapon-induced inhibition with pantothenate, beta-alanine, and l–pantoic acid. When these compounds were supplied to cucumber roots simultaneously with dalapon during the test period (36–60 hours) only l–pantoic acid gave any indication of reversing the dalapon inhibition. One of the difficulties was that the compounds were toxic when used in molar concentrations greater than the dalapon concentration. In a second experiment, cucumber seedlings were germinated and grown in buffer for 28 hours, transferred to 10^{-3}M pantoate (potassium salt) for 4 hours, and then to 10^{-3}M beta-alanine for 4 hours. For the remainder of the test (36–60 hours) the seedlings were transferred to a solution of 2×10^{-3}M dalapon. Pretreatment with l–pantoate and beta-alanine in series was able to cause a small but statistically significant alleviation of the dalapon-induced inhibition (Table 1).

Similar experiments were performed with corn. No system was devised which reversed the dalapon-induced inhibition in corn roots.

Higher green plant cells contain relatively little pantothenate or coenzyme A (of which pantothenate is a component) compared with yeast or microbial cells (17). Inhibition of pantothenate synthesis could be of less physiological importance in higher green plants than in these other species with respect to the total herbicidal activity.

Table 1. Effect of pre-treatment with 10^{-3}M *l*–pantoate and 10^{-3}M B–alanine on subsequent root elongation.

Pre-treatment	Growth period	Root elongation
Buffer	Buffer	21.2 mm.
Buffer	Dalapon	8.9
l–pantoate 4 hr + B–alanine 4 hr	Buffer	20.8
l–pantoate 4 hr + B–alanine 4 hr	Dalapon	12.1
LSD 5%, 2.9		

SUMMARY

1. The elongation of the primary root of corn was inhibited by concentrations of dalapon greater than 10^{-3}M. Cucumber roots were more sensitive to dalapon, with inhibition at 5×10^{-4}M. The dosage required for 50% inhibition was approximately 5 times greater for corn than for cucumber roots.

2. The pH of the ambient solution influenced the activity of dalapon. With corn roots, the growth response was proportional to the concentration of undissociated molecules. This response to pH was in part due to greater uptake at lower pH levels. The relationship between uptake and pH was not found to be as nearly linear as that between growth response and pH.

3. Inhibition of root elongation commenced after 4 hours exposure to dalapon. Root elongation ceased after 12 hours exposure. Phytotoxic amounts of dalapon could be absorbed from 5×10^{-3}M solutions by corn roots in 1 hour.

4. Concentrations of dalapon which inhibited root elongation had no effect on oxygen uptake by either corn or cucumber roots in a 6 hour period. Oxygen uptake by corn root mitochondria was not affected by similar concentrations of dalapon. Phosphate esterification was reduced by less than 25%.

5. The increment of oxygen uptake which accompanies aging of carrot root slices was inhibited by 5×10^{-3}M and completely suppressed by 7.5×10^{-3}M dalapon. Basal respiration was not affected.

6. Uptake of P^{32} as the H_2PO_4-ion by excised corn roots was reduced 50% by 12 hours pretreatment with 5×10^{-3}M dalapon. At a concentration which caused about the same growth response, DNP caused a 20% reduction in P^{32} uptake.

7. The inhibition of growth of cucumber root by dalapon could be reversed only to minor extent with pantothenic acid, l–pantoic acid, or beta-alanine. While pantothenic acid synthesis may be one site of dalapon action, the total herbicidal activity must be a result of still other effects.

8. On the basis of these findings, it is suggested that dalapon does not interfere with respiration or the production of metabolic energy, but with its utilization.

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Control of Barnyardgrass (*Echinochloa crusgalli*) in Western Irrigated Cotton[1]

J. H. Miller, H. M. Kempen, J. A. Wilkerson, and C. L. Foy[2]

Introduction

Improved methods of weed control through use of herbicides offer important means of reducing production costs and labor requirements in irrigated cotton. The use of pre-emergence herbicides has proved to be an effective tool for annual-weed control in cotton in the humid southeastern cotton region of the United States (5, 8). Research in Arizona (1, 3) has shown that certain herbicides applied at layby (immediately after the last cultivation) are effective for the control of late-season annual weeds in cotton.

To date chemical weed control has had only limited usage in California cotton. Hand-hoeing is the principal supplement to cultivation for early-season weed control. Flame cultivation, while proved to be an effective method for mid to late season annual-weed control (2, 4, 9, 10), is used to a very limited extent. Because of the need for better weed control (6) the experiments in this study were initiated to evaluate herbicides for annual-grass control in furrow-irrigated cotton where rainfall is generally insignificant during the growing season.

Materials and Methods

Investigations were conducted annually at pre-emergence and layby over a four year period in the San Joaquin Valley of California. The experiments were located on Delano clay loam in 1955 and 1956 and on Hesperia fine sandy loam in 1957 and 1958. In 1955 and 1956 they were conducted on a grower's field heavily infested with barnyardgrass (*Echinochloa crusgalli*), whereas in 1957 and 1958 they were conducted at the U. S. Cotton Field Station on land artificially infested with barnyardgrass seed at the rate of 75 to 100 lb/A.

For pre-emergence studies the weed seeds were planted prior to seedbed preparation, whereas for the layby studies they were sown and covered by cultivation just prior to herbicide application. Each year Acala 4–42 cotton seed was planted on 40-inch centers near mid-April. Planting, fertilization, irrigation, and cultivation prior to herbicide application were standard except for (1) use of highbed planting for the pre-emergence studies and (2) control of the irriga-

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tion water immediately after application of layby herbicides to give complete wetting of the cotton beds.

The herbicides studied were 3–(*p*–chlorophenyl)–1,1–dimethylurea (monuron) and 3–(3,4–dichlorophenyl)–1,1–dimethylurea (diuron) at rates of 1 and 1.5 lb/A; and isopropyl *N*–(3–chlorophenyl) carbamate (CIPC), 2–chloro–*N*,*N*–diallylacetamide (CDAA), and the sodium salt of *N*–1–naphthylphthalamic acid (NPA) at 6 and 9 lb/A.

The pre-emergence treatments were made as 12-inch bands over the row on the day of planting or one day after planting. The treated bands were undisturbed for 60 days after herbicide application. In 1957 the normal practice of not irrigating after application of pre-emergence herbicides was compared to the practice of furrow irrigating immediately after application of the herbicides. The irrigation water was controlled so that the tops of the beds were thoroughly wetted in order to activate the herbicides. However, rainfall occurred after the plots were irrigated.

The layby treatments were directed broadcast applications made during the last half of June (early layby) or last half of July (normal layby). The plots were irrigated as soon as possible after treatment, generally on the day after herbicide application, and except for irrigation were not disturbed until harvest. In 1955 duplicate sets of replicated plots comparing the same herbicides under different water management programs were initiated. One set of plots was irrigated according to grower practice and compared with plots where water was controlled to wet the surface soil over the entire bed.

Each date of application was considered as an experiment. The experiments were arranged in a randomized block and treatments were replicated four times each year. Plot size was 0.02 acres. Herbicides were applied in water at 50 gpa with a tractor-mounted sprayer. All plots were machine-harvested for yield.

The data taken were the following:

1. Weed-control ratings, made approximately 60 days after pre-emergence applications and at cotton harvest after layby applications. The ratings were based on a range of 0 to 10, zero indicating complete ground cover and 10 complete absence of weeds.
2. Yield of machine-picked seed cotton.
3. Quality of lint, as determined by grade, color, and staple or fiber length. At harvest, 25-lb samples of seed cotton were collected and ginned separately. In the ginning operation two-lb samples of cotton lint were collected and submitted to the U. S. Classing Office, Bakersfield, for evaluation.

Analysis of variance was used to test the weed-control ratings and seed-cotton yields.

In 1957 and 1958, after the cotton was harvested, barley was planted as an indicator plant to test for residual herbicide toxicity. In 1957 the area was disked to a depth of six inches twice with a heavy off-set disk plow, whereas in 1958, it was plowed 12 inches

deep with a moldboard plow and disked twice with an off-set disk plow. Then the area was planted with 80 lb/A of Mariout barley, furrowed, and irrigated. Injury ratings were recorded approximately 60 days after planting. The barley was plowed down in late February before yields could be measured.

RESULTS AND DISCUSSION

Herbicides applied pre-emergence to cotton.

In evaluating the pre-emergence herbicide data (Table 1) the difference in rainfall after herbicide application for each of the four years must be considered. In 1955, 1.5 inches of rain fell; 0.8 inch fell within one week after application. No rain fell after the 1956

Table 1. Pre-emergence herbicides for the control of barnyardgrass in cotton.

Herbicide	Rate lb/A	Weed rating[a]			
		1955	1956	1957	1958[b]
None (control)	0	0	6.3	0	1.6
NPA	6	9.5[c]	5.9	7.0	–
	9	10.0[d]	5.3	6.5	–
CIPC	6	7.5	5.3	8.5	4.3
	9	9.0	6.8	9.0	5.0
Monuron	1	5.5	5.3	6.5	5.0
	1.5	4.0	5.2	6.5	7.9[c]
Diuron	1	7.5	–	4.5	7.0
	1.5	7.0	–	5.0	8.0
CDAA	6	–	6.3	8.5	2.0
	9	–	7.1	9.0	2.3
LSD.05		3.6	ns	2.5	3.4
.01		5.8	ns	3.2	4.6
		Inches of rainfall after herbicide application			
		1.5	0	0.7	1.1

[a]0 = no control; 10 = complete control. Rated 60 days after treatment.
[b]Cotton irrigated up; rain occurred after irrigation.
[c]Cotton stand reduced.
[d]Cotton stand and yield reduced.

herbicide application. In 1957 no rain occurred until two weeks after application, when 0.7 inches fell in two showers. In 1958, because drying conditions were unfavorable to germination, the cotton was furrow-irrigated two weeks after planting in order to obtain a stand. One week after irrigation 1.1 inches of rain fell.

Weed control was variable both within and among years; however, real differences were apparent. In 1955 under environmental conditions favorable for the activation of herbicides, only NPA and the high rate of CIPC gave highly satisfactory weed control. Cotton stands were reduced by both rates of NPA, but seed-cotton yields were reduced only by 9 lb/A. Although other treatments were statistically better than the untreated plots the weed control obtained was not adequate to eliminate the need for additional early weed control measures.

Because no rain fell after herbicides were applied in 1956, none of the herbicides provided noticeable weed control. In 1957 with 0.7 inch of rain two weeks after herbicide application, all herbicide treatments gave significantly better weed control than the control plots, but only CIPC and CDAA provided weed control sufficient to eliminate hoeing. Under the conditions that existed in 1958, none of the herbicides provided satisfactory weed control even though several treatments were significantly better than the control plots.

The effect of an immediate furrow irrigation after application of pre-emergence herbicides was measured in 1957 (Table 2). Seven-

Table 2. The effect of furrow-irrigation on the efficiency of herbicides applied as pre-emergence treatments in cotton, 1957[a].

Herbicide	Rate lb/A	Not irrigated		Irrigated	
		Weeds/sq ft	Cotton/ft	Weeds/sq ft	Cotton/ft
None (control)	0	4.9	2.7	12.1	2.4
NPA	6	1.5	2.9	1.0	.5
	9	1.7	2.7	.7	.4
CIPC	9	.7	3.2	2.6	2.2
Monuron	1	1.7	3.0	.6	1.8
	1.5	1.6	2.8	.5	1.7
Diuron	1	2.8	2.8	2.0	2.6
	1.5	2.1	3.1	.7	2.8
CDAA	6	.8	2.9	5.6	2.5
	9	.8	2.7	7.5	1.8

[a]Treatments evaluated 60 days after application. All plots received rainfall 10 days (0.3 in.) and 13 days (0.4 in.) after treatments.

tenths inch of rain fell after herbicide application, but none until two weeks after irrigation. The performance of herbicides was altered and emergence problems, both from soil crusting and seedling diseases, were increased by furrow irrigation. With irrigation weed control was increased with monuron, diuron and NPA, but with the possible exception of diuron, the toxicity to cotton was likewise increased. CDAA and CIPC provided definitely less satisfactory weed control when irrigation followed herbicide application. The 1957 data were supported by the 1958 results when irrigation was necessary to induce cotton germination.

The study shows that pre-emergence herbicides in furrow-irrigated cotton depend on favorable water relationships for success. The fact that such conditions are seldom achieved precludes the use of pre-emergence herbicides in far western furrow-irrigated cotton unless other methods of application are found. Preliminary investigations in California (7) have showed that incorporation of certain herbicides in moist soil during cotton planting may insure weed control without rainfall.

Herbicides applied at layby in cotton.

Comparison of the two dates of application shows that early-layby control plots had much heavier weed populations than normal-layby control plots (Table 3). This was true regardless of whether

Table 3. Layby herbicides for the control of barnyardgrass in cotton.[a]

Herbicide	Rate lb/A	Early-layby weed control rating for				Normal-layby weed control rating for			
		1955	1956	1957	1958	1955	1956	1957	1958
None (control)	0	2.6	4.1	4.5	6.9	5.9	6.7	6.2	8.0
NPA	6	6.8	6.2	7.4	—	9.0	7.2	9.1	9.1
	9	6.6	5.7	7.7	—	9.1	7.5	8.0	8.4
CIPC	6	—	6.9	—	7.8	—	7.8	—	8.8
	9	—	7.4	7.5	8.4	—	8.3	6.7	9.0
Monuron	1	8.6	8.5	7.1	9.1	6.9	8.3	7.7	9.6
	1.5	8.5	8.9	8.8	8.8	7.4	9.0	8.9	9.5
Diuron	1	7.3	7.0	7.8	9.0	9.0	8.7	8.6	9.6
	1.5	8.8	8.0	8.6	9.1	9.1	8.0	9.4	9.6
CDAA	6	—	6.8	5.2	—	—	7.8	6.2	8.5
	9	—	7.4	4.6	—	—	7.9	7.3	8.6
LSD .05		.9	1.6	1.6	1.3	2.4	ns	1.8	1.0
.01		1.2	2.1	2.2	1.8	ns	ns	2.4	ns

[a] 0 = no control; 10 = complete control. Treatments evaluated immediately after harvest (late November).

the weed populations were natural (1955 and 1956) or artificially created by planting (1957 and 1958). The less dense weed population of the normal layby date undoubtedly was due to "closing in" of cotton growth over the rows, which shaded much of the soil. Because of the difference in weed populations, a greater difference was shown between treated and untreated plots at the early date even though the weed-control evaluations for treated plots on the two dates were comparable.

All herbicides applied at early-layby, except CDAA in 1957, improved weed control significantly over the control plots. Among the herbicides, monuron and diuron provided the most satisfactory weed control. When applied at normal-layby, diuron, NPA and monuron controlled weeds more consistently than CIPC or CDAA. Monuron and diuron were equally effective for weed control at the early-layby date. Diuron tended to be superior to monuron at the normal-layby date. One marked difference between monuron and diuron was that surviving weeds in diuron-treated plots were generally in the cotton row, whereas the weeds in monuron-treated plots were usually in the furrows. Differences probably were due to solubility and absorption differences between the herbicides.

Comparison of water-management practices after herbicide application in 1955 clearly showed that the herbicides failed to control weeds unless the entire surface of the bed was wet (Figure 1).

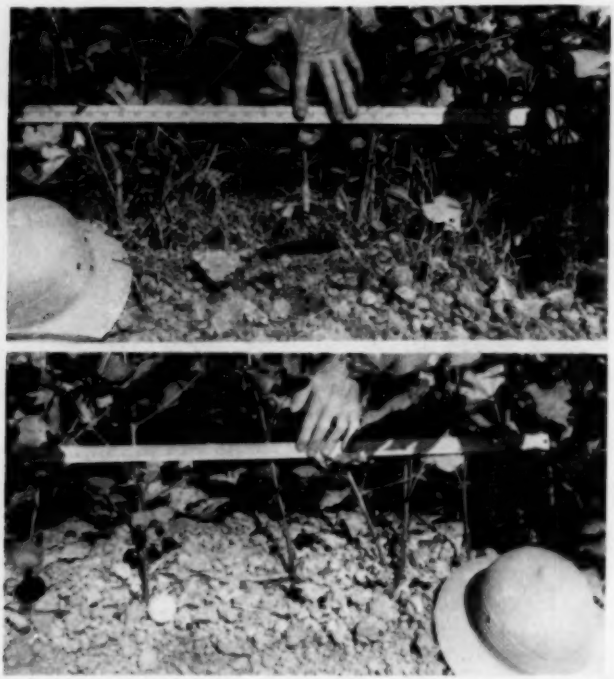

Figure 1. The effect of water management upon herbicides applied in cotton at layby.
(Top) Diuron applied at 2 lb/A followed by standard irrigation practice. Note the grass in the drill row where water had not advanced across the top of the bed. Grass in foreground is dead.
(Bottom) Diuron applied at 2 lb/A followed by irrigation in which the top of the beds were completely wet. Note the absence of grass in the drill row.

Yield of seed cotton was not altered with any of the layby herbicides during the course of this study. The lack of yield differences would indicate (1) that the weeds that developed on the control plots after mid-June failed, with ample moisture and nutrients for the growth of both grass and cotton, to provide competition sufficient to lower yields, or (2) that herbicide injury to cotton, even though not apparent, was sufficient to counterbalance the competitive effects of weeds.

Grass trash is commonly considered as being difficult to remove with the cleaning equipment in the ginning process. The fact that considerable additional cleaning equipment was added to the Field Station cotton gin before the 1957 harvest, however, may well have influenced the grade-index data (Table 4) of 1957 and 1958. With

Table 4. Effect of herbicides applied at layby upon grade index[a] of cotton lint.

Herbicide	Rate lb/A	Grade index for early layby				Av	Grade index for normal layby				Av
		1955	1956	1957	1958		1955	1956	1957	1958	
None (control)	0	95.5[b]	98.5[c]	94.0	94.5	95.6	100.5	99.0	98.0	98.0	98.9
NPA	6	—	100.0	95.0	—	97.5	—	100.0	95.5	98.0	97.8
	9	98.5[c]	100.0	94.0	—	97.5	100.0	100.0	97.0	98.0	98.8
CIPC	6	—	100.0	—	98.0	99.0	—	100.0	—	99.0	99.5
	9	—	100.0	94.5	97.5	97.3	—	99.0	96.5	100.0	98.5
Monuron	1	—	100.0	95.0	99.0	98.0	—	100.0	95.5	100.0	98.5
	1.5	100.0	100.0	96.0	98.5	98.6	100.5	100.0	97.0	100.0	99.4
Diuron	1	—	100.0	94.5	97.5	97.3	—	98.0	100.0	100.0	99.3
	1.5	98.5	100.0	96.5	96.5	97.9	101.0	100.0	97.0	100.0	99.7
CDAA	6	—	100.0	94.0	—	97.0	—	97.0	94.5	97.0	96.2
	9	—	100.0	92.5[c]	—	96.3	—	98.5	95.0	100.0	97.8

[a]Grade index: Middling (M) = 100, strict low middling plus (SLM +) = 96, strict low middling (SLM) = 94, low middling (LM) = 85. In addition to grade loss, bales reduced in grade because of grass are docked 4 cents/lb.
[b]75% of samples reduced one grade because of grass.
[c]25% of samples reduced one grade because of grass.

the early-layby application the grade index of cotton lint from control plots was two points, or about one-half grade, lower than the treated plots. Grade losses were encountered with lint from control plots in 75 percent of the samples in 1955; 25 percent in 1956; none in 1957, but 75 percent carried a notation "a trace of grass"; and none in 1958. Among the treated plots, grade reductions were found in 25 percent of the lint samples from plots treated with NPA in 1955, and 25 percent of the plots treated with the higher rate of CDAA in 1957. No other grade losses were found.

Grade index of samples taken from normal layby plots was not altered by treatment. None of the samples were classified as "grassy". After normal layby, very little grass attained sufficient height to become incorporated into the cotton lint in the mechanical harvesting process. Thus weed control at this date altered neither yield nor quality of cotton, but to a large degree did prevent reseeding of the soil with grass seed.

Residual toxicity of herbicides applied in cotton.

Only monuron and diuron left sufficient residue in the soil to be toxic to winter-sown barley (Table 5). Residual toxicity occurred

Table 5. Residual toxicity of herbicides applied at different times in cotton, on winter-sown barley planted after disking (1957) or deep plowing (1958)[a].

Herbicide	Rate lb/A	Rating, 1957 treatments			Ratings, 1958 treatments		
		Pre-emergence	Early layby	Normal layby	Pre-emergence	Early layby	Normal layby
None (control)	0	2.4	.4	.2	0	0	0
NPA	6	1.3	.8	.7	–	–	0
	9	.8	.6	.6	–	–	0
CIPC	6	1.5	–	–	0	0	0
	9	1.7	1.3	1.0	0	0	0
Monuron	1	2.1	6.2	5.6	0	1.0	1.0
	1.5	2.2	6.8	6.9	0	1.5	2.5
Diuron	1	3.2	3.6	4.0	0	.5	.5
	1.5	2.7	6.2	5.2	0	.5	.5
CDAA	6	1.6	.7	.8	0	0	0
	9	2.0	.2	.9	0	0	0

[a]0 = normal; 10 = complete kill. Treatments rated about February 1 each year.

only where these herbicides were applied at the layby dates. The lack of residual toxicity from pre-emergence treatments was partially attributed to the greater period after the treatment, but was largely due to the fact that the pre-emergence treatments were 10-inch band treatments.

Residual toxicity from monuron and diuron treatments at both layby dates were obvious under each tillage practice. Deep plowing, to a depth of 12 inches greatly reduced residual toxicity in 1958. The reduced toxicity was undoubtedly due to additional dilution

of the herbicide residue by deep tillage. Injury to winter-sown barley was greater with monuron than with diuron.

SUMMARY

A four year study was conducted to determine the effect of NPA, CIPC, monuron, diuron, and CDAA, applied at different dates, on barnyardgrass control and yield and quality of cotton.

Pre-emergence herbicide applications controlled barnyardgrass in varying degrees, depending on post-application rainfall.

Layby applications of several herbicides in late-June reduced barnyardgrass without loss of cotton yield and in many cases improved quality of lint. Similar applications applied in late July performed in a similar manner except that the weed problem was less acute, and hence, grades of lint were not altered. Among the herbicides tested at layby monuron and diuron provided the most consistent economical weed control, but they also left undesirable residues in the soil.

Water management had a marked effect upon the performance of layby herbicides in furrow-irrigated cotton. Unless the cotton beds were well wetted following herbicide application poor weed control resulted.

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The Influence of *N*–(3,4–dichlorophenyl)methacrylamide on Early Growth and Development of Cotton[1]

S. W. Bingham and W. K. Porter, Jr.[2]

N–(3,4–dichlorophenyl)methacrylamide (hereafter referred to in this paper as DCMA) is a relatively new compound in the field of herbicides. However, several weed control workers (personal communication) have suggested that DCMA may be of value for control of crabgrass (*Digitaria* spp.), brachiaria (*Brachiaria* spp.), pigweed (*Amaranthus* spp.), morning glory (*Ipomoea* spp.), cocklebur (*Xanthium* spp.), and goosegrass (*Eleusine indica*), common weeds found in cotton.

Porter et al. (4) and Harris et al. (1) noted that cotton was stunted by DCMA when applied over the top of the plants. They indicated that later season compensatory growth overcame this stunting. Porter et al. proposed that DCMA for post-emergence weed control could be semi-directed under the cotyledons of young cotton without injury to the plants.

The purpose of this study was to determine the nature of this stunting during early development of cotton.

Methods and Materials

Cotton (*Gossypium hirsutum* L. Var. Deltapine 15) was planted in flats of sandy loam soil in the greenhouse and allowed to develop for 5 to 6 days. At this time, some of the flats were treated with a commercial emulsifiable concentrate of DCMA at 3 lb/A broadcast over the top of the plants. Treated and untreated plants were immediately transferred to growth rooms at 30°C and approximately 500 foot candles of light from a combination of fluorescent tubes and incandescent bulbs. In studies involving the first true leaf, the plants were treated on the tenth to eleventh day after planting and transferred to growth rooms at that time.

Growth was measured in terms of fresh weight, dry weight, and cell enlargement. Total nitrogen was determined by a micro-Kjeldahl procedure (2).

For studies involving hypocotyl sections, cotton seed were planted in flats of soil and placed in a 25°C constant temperature darkroom with high humidity. When the hypocotyls were 2 to 3 cm long, 5.8 mm sections were cut beginning 3 mm below the crook. The sections were floated for 3 hours on glass distilled water containing 1 mg/l of $MnSO_4 \cdot H_2O$. Ten sections were then placed in 3 ml of test solution of various rates of technical DCMA, indoleacetic acid (IAA), or both DCMA and IAA. Except for the above modifications the procedure described by Nitsch and Nitsch (3) for the first internode test was used. The sections were measured to the

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nearest 0.1 mm using a binocular dissecting microscope and ocular micrometer.

These manipulations were carried out under a safelight (3) which gives a light having mostly the wavelength of the 546 mu mercury band. This light did not inhibit the growth of the hypocotyl and was phototropically inactive.

For root elongation studies, cotton seed were placed on the surface of wet soil and covered with wet cheesecloth. The seed were allowed to germinate in the dark at 25°C and high humidity. After 3 days the seedlings were grouped into batches with uniform length of roots. The roots were measured and placed in the test solution through a hole in foil paper. The green safelight was also used while making these manipulations. Technical DCMA and IAA were also used in these studies. All test solutions contained a final concentration of 5 mM $Ca(NO_3)_2$, 6.7 mM KH_2PO_4, and were adjusted to pH 5.9 with HCl or KOH. The roots were allowed to elongate for 24 hours and the lengths measured to the nearest 1 mm.

Results and Discussion

Under growth-room conditions, cotyledons yielded an S-shaped growth curve (fresh weight). In preliminary experiments it was observed that the time of treatment giving the greatest reduction of cotyledon weight was at 5 to 6 days after planting, or at the onset

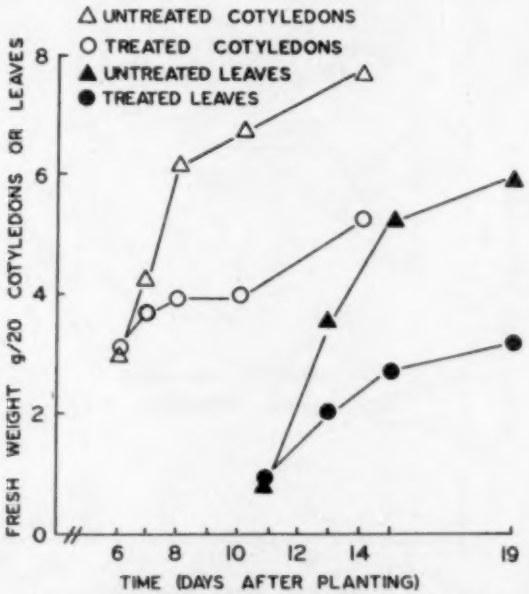

Figure 1. The influence of DCMA on the growth of cotyledons and first true leaves.

of most rapid growth. The rate and amount of development was markedly reduced by DCMA (Figure 1). The reduction was rather uniform with no observable malformations of the tissue. Anatomical examinations indicated that cell enlargement was reduced to the same extent as fresh weight.

The effect of DCMA on the elongation of the first internode and on the expansion of the first true leaf is shown in Table 1. The

Table 1. The effect of DCMA at three cotyledon stages on the per cent inhibition of the first internode and of expansion of the first true leaf.

Age of plants at treatment (days after planting)	Per cent inhibition, days after treatment			
	7		12	
	Internode	Leaf	Internode	Leaf
6	76	61	53	35
10	54	34	31	26
14	−16	18	−18	8

time of treatment giving the greatest reduction of internode elongation coincided with that which caused the greatest inhibition of cotyledon development. The first internode began to elongate on about the tenth day after planting. Thus, any later treatments would have less effect on the total length of this internode; how-

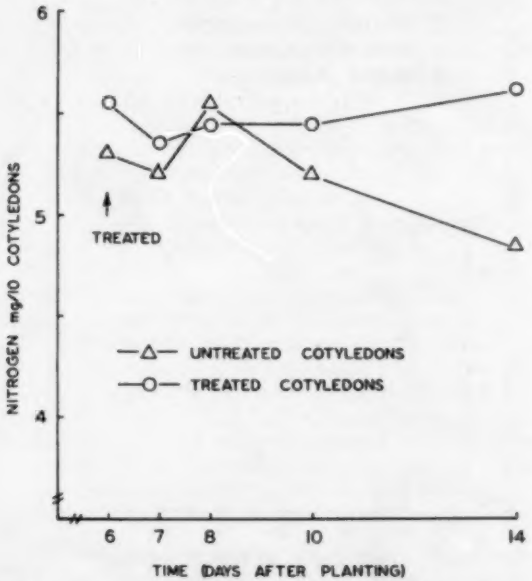

Figure 2. The influence of DCMA on the nitrogen content per cotyledon.

ever, when the second internode was considered, DCMA treatment on the fourteenth day had a pronounced effect. The plants began to recover on the fourth to sixth day after treatment and marked recovery was noted by the measurements made on the twelfth day.

True leaf expansion was also temporarily reduced by DCMA. These results were very similar to that for internode elongation.

Cotton cotyledons contained quite high levels of nitrogen (Figure 2). The nitrogen content of untreated cotyledons decreased slightly during development indicating possibly some movement to other plant parts. Since the nitrogen content of treated cotyledons remained constant there was no evidence of movement to other parts for 8 days. Thus, combining the suppressing effect on growth and immobility of nitrogen compounds, treated cotyledonary tissue contained substantially more nitrogen than control tissue (Figure 3).

Similar studies were carried out for the first true leaf. The effect of DCMA on fresh weight (Figure 1) and cell size was similar to that obtained with cotyledons. In treated and untreated true leaves the nitrogen content increased as the weight of the leaves increased. Treated cotyledons or true leaves never reached the full size of the untreated; they tended to mature earlier and sometimes the cotyledons dropped off the plants at a much earlier stage, especially from treated plants left in the greenhouse.

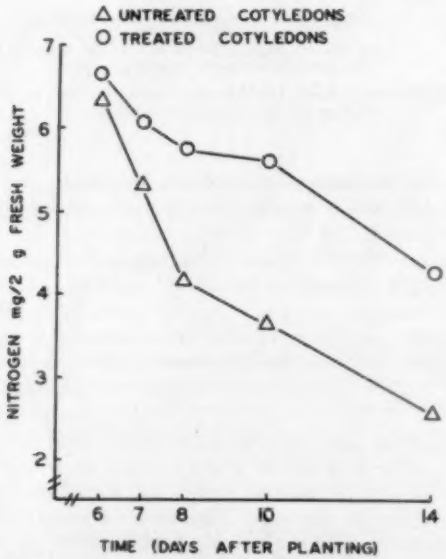

Figure 3. The influence of DCMA on the nitrogen content of cotyledonary tissue.

Since there was a large effect of DCMA on cell enlargement, it appeared advisable to study the effect on straight growth of hypocotyl sections. In control solutions the sections elongated approximately 30 per cent. The greatest stimulation obtained with IAA was with 10 ppm (Figure 4). This maximum stimulation by IAA was cut in half by an equal weight concentration of DCMA. DCMA alone had very little effect on growth of hypocotyl sections.

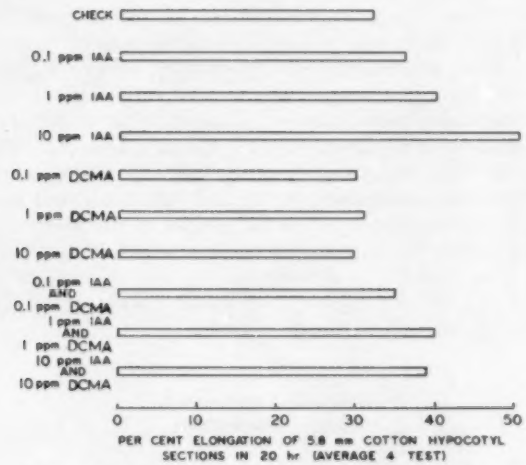

Figure 4. The effects of IAA, DCMA, and combinations of both on the elongation of hypocotyl sections.

Since roots contain optimum to above optimum concentration of auxin, any added auxin would thus act to reduce elongation of intact roots (Figure 5).

It appeared that DCMA could be acting as a weak auxin. In the case of the straight growth of hypocotyl sections, DCMA acted to reduce the stimulatory action obtained with IAA. Weak auxins compete with very active auxin to yield reduced growth of internode or coleoptile sections. In the case of roots, any added auxin should act to reduce elongation. These were the results obtained with DCMA.

DCMA treatment just prior to rain under field conditions gave essentially the same amount of weed control as when no rain followed treatment, but the cotton plants did not show the characteristic stunting normally observed. This lack of stunting was studied in the laboratory by washing off the herbicide at various intervals after treatment and observing the growth response (Figure 6). The effectiveness of DCMA in inhibiting growth of cotyledons increased

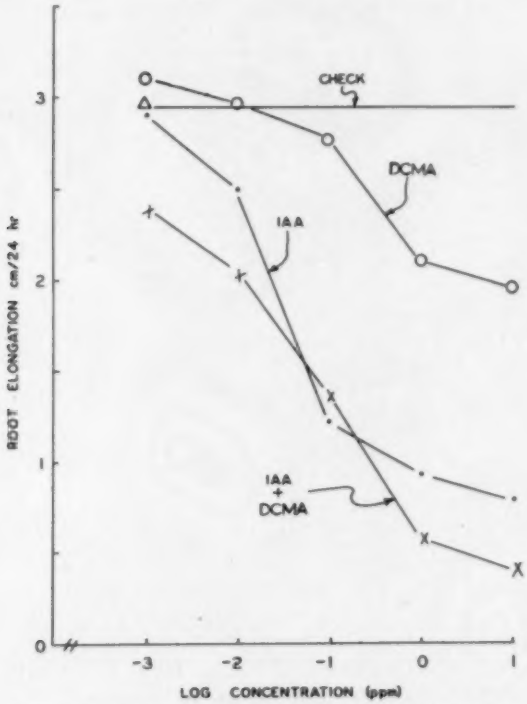

Figure 5. The effects of IAA, DCMA, and combinations of both on the elongation of intact primary roots.

with increasing periods of contact. Short intervals gave intermediate amounts of growth reduction. Maximum reduction obtained with no washing was approached closely by 3 hours of contact. Thus, it appeared that penetration into the cotyledonary tissue was quite rapid.

SUMMARY

N–(3,4–dichlorophenyl)methacrylamide (DCMA) reduced the rate of enlargement of cotyledons, the elongation of internodes, and expansion of true leaves. Maximum reduction of cotyledon weight was obtained when DCMA was applied at the onset of most rapid growth. Anatomical examinations of cotyledons and leaves indicated that the rate and amount of cell enlargement was uniformly reduced by DCMA.

Since the nitrogen content of treated cotyledons remained constant while that of untreated cotyledons decreased, DCMA appeared to immobilize somewhat the nitrogen in the cotyledons. In both

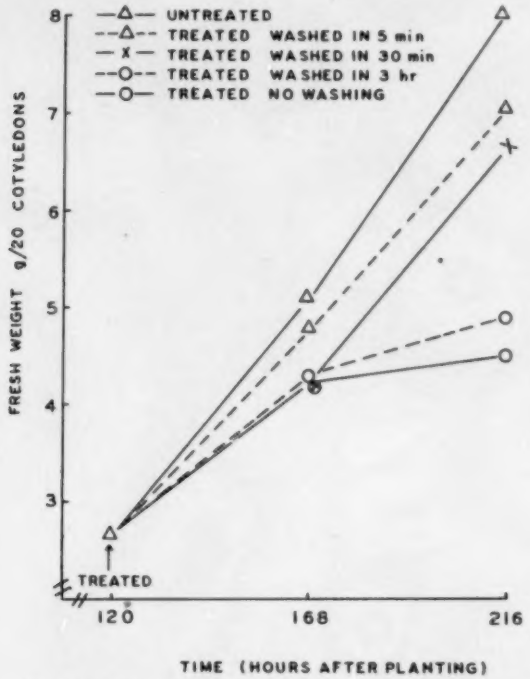

Figure 6. The effect of washing off the herbicide at various intervals on the growth response of cotyledons.

treated and untreated true leaves the nitrogen content increased as the weight of leaves increased.

The effects of DCMA on elongation of hypocotyl sections and intact primary roots was investigated and compared to that obtained with IAA. The greatest stimulation of hypocotyl sections obtained with IAA was cut in half by an equal weight concentration of DCMA. Both DCMA and IAA acted to reduce elongation of intact roots. These results suggested that DCMA could be acting as a weak auxin.

The effectiveness of DCMA for inhibition of growth of cotyledons increased with increasing periods of contact, maximum reduction having been approached closely in 3 hours.

ACKNOWLEDGEMENT

Acknowledgement is made to Dr. J. B. Baker for helpful advice relating to experimental work.

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The Activities of Certain Enzymes from Cotton Treated in Cotyledon Stage with N–(3,4–dichlorophenyl) methacrylamide[1]

S. W. Bingham and W. K. Porter, Jr.[2]

According to Bonner (3), at least three different oxidase systems may be involved in the final step of the transfer of electrons to oxygen. Each of the terminal oxidases has been shown to occur in many plants and in many relative combinations.

Several workers have suggested that some plants use one terminal oxidase system and later change to another (1, 4, 9). For instance, Mayer (9) reported that lettuce seedlings at first have a high ascorbic acid oxidase activity. Later, ascorbic acid oxidase activity decreased and other oxidase systems increased in amount of activity.

Mapson (8) showed that reduced glutathione and ascorbic acid act as respiratory carriers in an enzyme system which transfers hydrogen to oxygen from substrates of triphosphopyridine nucleotide-linked dehydrogenases. Several workers have reported more positive evidence of the function of ascorbic acid oxidase as a terminal enzyme. For example, James (5) and James and Boulter (6) observed that in 10- to 17-day old barley roots ascorbic acid oxidase was the only one of the three terminal oxidases that they were able to demonstrate.

On the other hand, Thimann et al. (11) have sought to establish that ascorbic acid oxidase is not of major importance in many tissues as a terminal enzyme on the basis of low affinity of the enzyme for oxygen.

The evidence as reviewed at this time still leaves it uncertain whether ascorbic acid oxidase is of importance as a terminal oxidase. Newcomb (10) showed this enzyme to be associated with the cell wall fraction and ascorbic acid oxidase activity increased with an increase in cell size of tobacco pith stimulated by indoleacetic acid. The function of this enzyme could be associated with some specific role in the growth of the primary wall which may control cell enlargement.

Some of the properties of ascorbic acid oxidase of cotton tissues are reported in this study.

Methods and Materials

Cotton was grown in flats of soil in the greenhouse, treated with *N*–(3,4–dichlorophenyl)methacrylamide (hereafter referred to in this paper as DCMA) and transferred to growth rooms as described previously (2).

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Respiration of tissue slices.

Gas exchange determinations were carried out by conventional Warburg methods (12). Several cotyledon or leaf slices weighing a total of 325 to 375 mg fresh weight were placed in a Warburg vessel which contained 2 ml of a pH 6 buffer composed of potassium phosphate at 0.05 M and sucrose at 0.02 M. Duplicate flasks were employed and the test was repeated.

Estimation of enzyme activity.

Preparation of homogenates. Two grams of tissue slices were placed in an Omni mixer followed by 10 ml of appropriate buffer solution. The tissue was ground 10 minutes at a temperature below 5°C. The vessel was rinsed with buffer and the volume made up to 30 ml. There were only a few whole cells in the crude homogenate and these were principally epidermal and fiber cells. The homogenates were used immediately after preparation for enzyme assays.

Ascorbic acid oxidase. A 3 ml aliquot of homogenate was added to the main compartment of the Warburg flask and 0.5 ml of 0.12 M ascorbic acid was placed in the side arm. All of the ingredients were prepared in 0.05 M potassium phosphate buffer which was adjusted to pH 6 with KOH. The ascorbate was tipped in following equilibration.

Polyphenyloxidase. A 3 ml aliquot of homogenate was placed in the main compartment of a Warburg vessel and 0.5 ml of 0.12 M catechol was added to the side arm. The homogenate and catechol were prepared in 0.05 M potassium phosphate buffer which was adjusted to pH 6. After equilibration the catechol was tipped into the homogenate.

Cytochrome oxidase. A 3 ml aliquot of homogenate was added to the main compartment of the double arm Warburg flask. One-half ml of 2×10^{-4} M cytochrome c and 0.5 ml of 0.12 M *p*–phenylenediamine were placed in separate side arms. These solutions were adjusted to pH 7 just prior to addition to the flask.

RESULTS AND DISCUSSION

Respiration of tissue slices.

The short time effects of DCMA on cotyledons were first evaluated. Oxygen uptake immediately after treatment was determined. A second test involved floating cotyledon discs on technical DCMA solutions at 10 ppm for 1 and 6 hours before determining oxygen uptake. From these studies, there appeared to be no immediate effect of DCMA on total oxygen uptake.

The respiration of cotyledonary tissue was determined at various intervals from zero to eight days after treatment (Figure 1). Respiration was declining quite rapidly during the period of most rapid growth (2). Since enzymes are considered nitrogenous, it is generally thought advisable to express enzyme activity on protein nitrogen. On a nitrogen (and per cotyledon) basis, respiration was only slightly

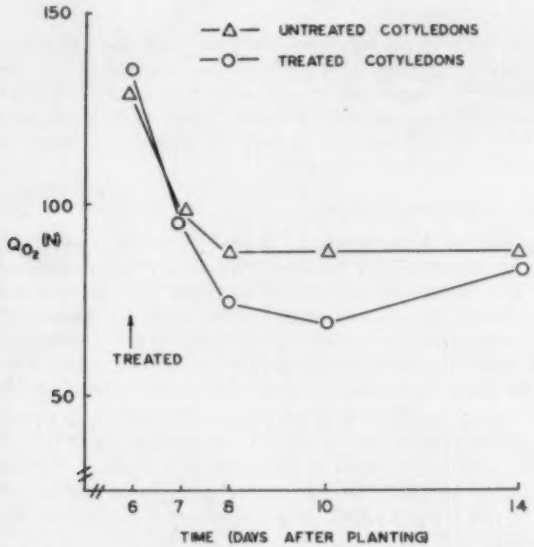

Figure 1. Oxygen uptake of untreated and treated cotyledon tissue at various time intervals after treatment. $QO_2(N)$ is ul oxygen uptake/mg nitrogen/hr.

less on the second to fourth day for treated as compared to untreated tissue. However, respiration was markedly higher for treated tissue on a fresh weight basis (Figure 2). On the eighth day after treatment, oxygen uptake by treated tissue was 64 per cent higher than by untreated control tissue. Since respiration was only slightly reduced on a nitrogen basis and each cotyledon contained approximately the same amount of nitrogen, it appeared that DCMA reduced growth without any substantial effect on total respiration.

The small effects of DCMA on respiration of true leaf tissue were similar to that for cotyledon tissue (Figure 3). It appeared from several tests that the slight reduction may have occurred earlier after treatment in the case of true leaves and recovery also appeared earlier.

Terminal oxidase activity in homogenates.

In tissue of higher plants, there are at least three terminal oxidase systems which may catalize the final step in the transfer of electrons from a substrate to oxygen (3). These involve cytochrome oxidase, polyphenyloxidase, and ascorbic acid oxidase. Thus, in order to study the effect of DCMA on terminal oxidation mechanisms, it appeared that relative activities of these oxidases should first be evaluated. This was studied in at least three ways: (1) using sub-

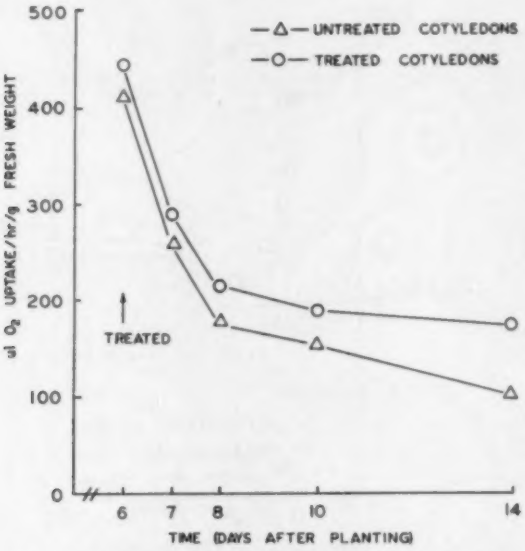

Figure 2. Oxygen uptake of untreated and treated cotyledon tissue at various time intervals after treatment, expressed on a fresh weight basis.

strates and measuring the rate of oxidation in homogenates, (2) using substrates and boiling the homogenate, and (3) using substrates and known inhibitors of the oxidase.

The results of tests employing certain substrates (ascorbate, catechol, and cytochrome c plus *p*-phenylenediamine) suggested that ascorbic acid oxidase was the predominant terminal enzyme in cotyledon tissue (Figure 4). Oxygen uptake was four to five times greater during the oxidation of ascorbate as compared to that for catechol in homogenates. No cytochrome oxidase activity was demonstrated using substrates.

Boiling the homogenate completely eliminated the oxidation of ascorbate in the homogenate. These results suggested that the oxidation of ascorbate was enzymatic. However, many enzymes are known to bring about the oxidation of ascorbate in homogenates of plant tissues. These enzymes can be divided into two groups: (1) those directly involved in catalyzing the oxidation of ascorbate and (2) those indirectly involved. According to Mapson (7), ascorbic acid oxidase is the only enzyme in the first category to be demonstrated in homogenates and tissue extracts. In the second group, polyphenyloxidase and cytochrome oxidase have been particularly important. Ascorbate has been used successfully as a reducing agent for cytochrome c and quinones in studies involving cytochrome oxidase and polyphenyloxidase, respectively. This aspect was studied

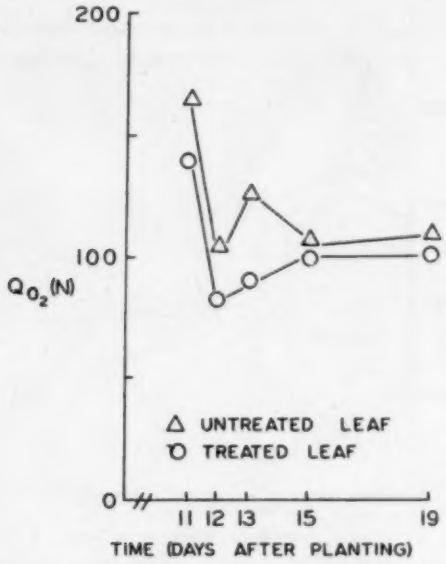

Figure 3. Oxygen uptake of untreated and treated first true leaf tissue at various intervals after treatment. $QO_2(N)$ is ul oxygen uptake/mg nitrogen/hr.

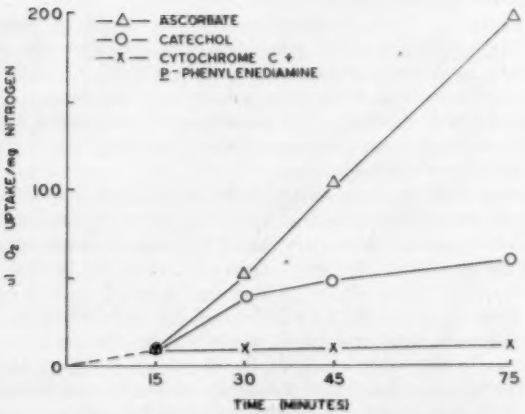

Figure 4. Oxidation of ascorbate, catechol, and cytochrome c plus *p*-phenylenediamine in homogenates of untreated cotyledon tissue. Substrates were tipped in at the 15 minute period.

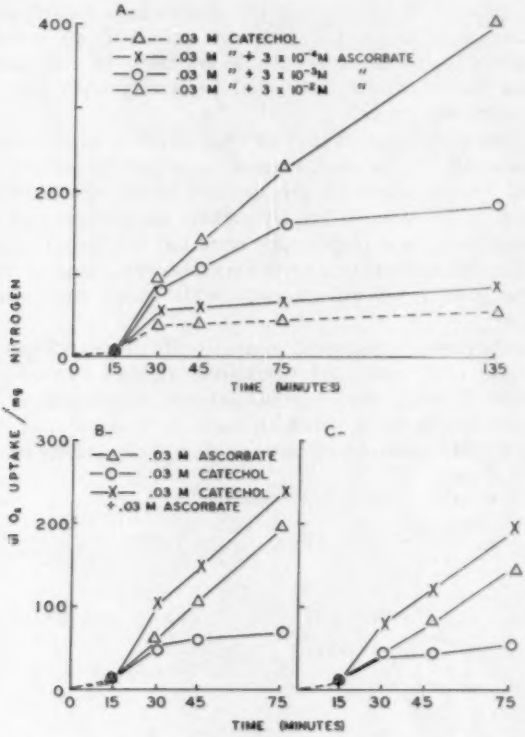

Figure 5. Oxidation of combined substrates, ascorbate and catechol, in homogenates of cotyledon tissue: A. Increasing amounts of ascorbate with catechol constant, B. Oxidation of optimum combinations as compared to either alone—untreated tissue, and C. Treated tissue.

using homogenates of cotyledon tissue and combined substrates, ascorbate and catechol (Figure 5).

There was a rate effect up to 3×10^{-2} M final concentration of ascorbate, this concentration being optimum. It appeared that ascorbic acid oxidase and polyphenyloxidase activities were additive and that ascorbate did not act as an electron source for reduction of quinones.

For further support of ascorbic acid oxidase activity in homogenates of cotyledon tissue, two inhibitors were used which effectively inhibit copper containing enzymes. Diethyldithiocarbamate (dieca) and thiourea gave 75 and 35 per cent inhibition of the rate of oxidation of ascorbate, respectively. The slight inhibition of respiration by carbon monoxide was not found to be light reversible.

In a review article by Mapson (7), it was indicated that ascorbic acid oxidase was associated with the cell wall in some tissues, mitochrondria in others, and soluble fractions in still others. The enzyme was found to be associated primarily with the cell wall material in cotton tissue.

All of these facts tend to suggest that ascorbic acid oxidase is the enzyme involved in the oxidation of ascorbate in cotton tissue homogenates. The function of this enzyme in the metabolism of the plant cell is unknown. It has frequently been suggested that this enzyme may serve as a respiratory terminal oxidase. It is also conceivable that this enzyme plays some specific, though as yet unknown, role in the growth of the primary wall which may control cell enlargement.

In the experiments discussed above, both untreated and DCMA-treated tissue were used and compared. DCMA caused a marked reduction in ascorbic acid oxidase activity varying from 25 to 75 per cent inhibition on a nitrogen basis at 4 days after treatment (Figure 6). DCMA reduced ascorbic acid oxidase activity in cotyledon

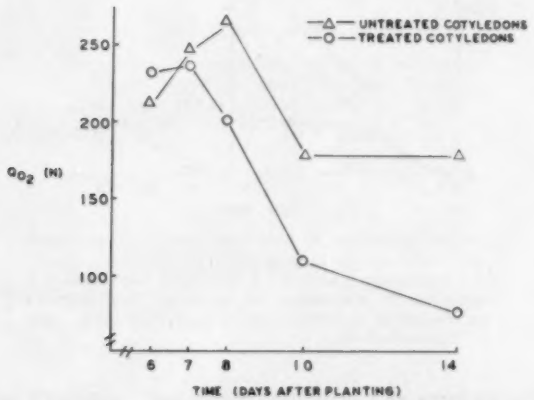

Figure 6. Oxidation of ascorbate in homogenates prepared at various intervals after treatment, expressed on a nitrogen basis.

tissue to the same extent as the effect on growth. In Figure 7 ascorbic acid oxidase activity is expressed on a fresh weight basis.

Ascorbic acid oxidase activity from true leaves was reduced to approximately the same extent on a nitrogen or fresh weight basis (Figure 8). The slight to moderate reduction in ascorbic acid oxidase activity was particularly noted at the second to fourth day after treatment and recovery noted on the eighth day.

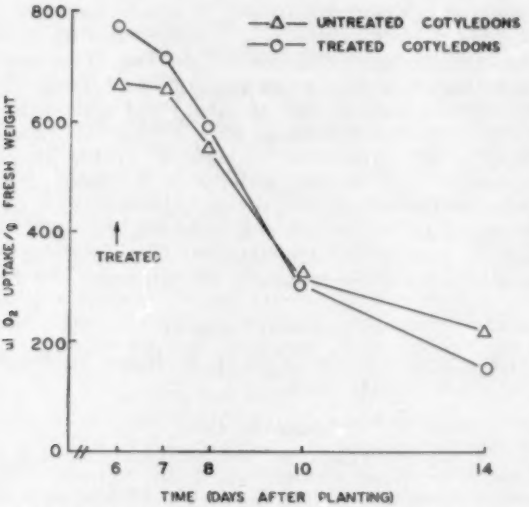

Figure 7. Oxidation of ascorbate in homogenates prepared at various intervals after treatment, expressed on a fresh weight basis.

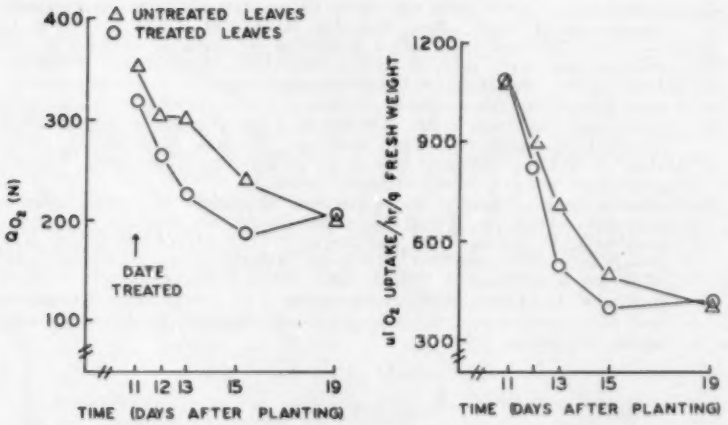

Figure 8. Oxidation of ascorbate in homogenates of first true leaf tissue, expressed on a nitrogen basis (left) and fresh weight basis (right).

SUMMARY

Respiration declined during development of cotton cotyledons. *N*-(3,4-dichlorophenyl) methacrylamide (DCMA) reduced growth with only a slight decrease in respiration per cotyledon.

The results employing substrates (ascorbate, catechol, and cytochrome c plus *p*–phenylenediamine) suggested that ascorbic acid oxidase was the predominant terminal oxidase. The rate of oxidation of ascorbate in homogenates was inhibited about 75 per cent by diethyldithiocarbamate and about 35 per cent with thiourea. Results with carbon monoxide as an inhibitor of respiration suggested little or no cytochrome oxidase in cotton tissue. DCMA reduced ascorbic acid oxidase activity in cotyledon tissue to the same extent as the effect on growth. Ascorbic acid oxidase was primarily associated with the cell wall fraction and completely inactivated by boiling the homogenate. Ascorbate did not act as an electron source for the reduction of quinones.

ACKNOWLEDGMENT

Acknowledgment is made to Dr. J. B. Baker for helpful advice relating to experimental work.

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Glycolic Acid Oxidase, Catalase and Peroxidase of Cotton Tissue[1]

S. W. Bingham and W. K. Porter, Jr.[2]

Glagett, Tolbert, and Burris (5) reported an enzyme of widespread occurrence in the green parts of many plants which catalyzed the oxidation of glycolic acid and l-lactic acid. Zelitch (14) isolated glycolic acid oxidase from spinach leaves and found it to be a flavoprotein with flavin mononucleotide as the prosthetic group. This work was later confirmed by Frigerio and co-workers (7).

Further work (10) revealed another enzyme, glyoxylic acid reductase, which catalyzed the reduction of glyoxylic to glycolic acid by reduced pyridine nucleotide. A possible hydrogen transfer system from substrate to oxygen was suggested to involve dehydrogenases, glyoxylic acid reductase, glycolic acid oxidase, and catalase.

Glycolic acid oxidase was not found in etiolated plants and was found only in plants which had been exposed to light. Benson and Calvin (2) have shown that glycolic acid builds up rapidly during photosynthesis and remains high in the light but the concentration falls in the dark. Zelitch (12, 13) permitted tobacco leaves to take up α-hydroxysulfonates, which are inhibitors of glycolic acid oxidase, and found that glycolic acid accumulated to ten times its normal level during photosynthesis. He suggested that respiration in sunlight may have taken place through the glycolic acid oxidase system. It was indicated that glycolic acid oxidase activity was sufficiently high for mediation of total oxygen uptake in tobacco leaves.

Catalase appeared important in this scheme in that glyoxylate in the presence of hydrogen peroxide will break down to formic acid, water, and carbon dioxide. If catalase was present, then, very little carbon dioxide was evolved (14). The ratio of carbon dioxide evolved to oxygen consumed was about zero to 0.3 for crude extracts of spinach as a source of glycolic acid oxidase.

The effect of chemical treatment of plants on the following catalase activity was studied by Landon (9). Watering plants with 0.1 M thiourea 24 hours prior to catalase determination reduced activity about 65 per cent. One molar ammonium sulfocyanate was required to give a similar amount of inhibition. Chlorate caused very little change in amount of activity.

Methods and Materials

Untreated cotton tissue and tissue treated with N–(3,4–dichlorophenyl)methacrylamide, hereafter referred to as DCMA, were obtained in a manner described previously (3). Homogenates were prepared using an Omni mixer (4). The supernatant of 1000 × g for 7 minutes at 0°C was used in these studies as the enzyme source.

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[2]Research Assistant and Associate Professor, respectively, Louisiana State University, Baton Rouge, Louisiana.

Glycolic acid oxidase. The enzyme was assayed by measuring the rate of oxygen uptake with l-lactate and glycolate as substrates (5). Conventional Warburg equipment was used. A 3 ml aliquot of supernatant was added to the main compartment and 0.5 ml of 0.14 M l-lactate or glycolate was placed in the side arm. A small piece of folded filter paper and 0.15 ml of 20 per cent KOH was placed in the center well. After equilibration at 25°C the lactate or glycolate was tipped into the supernatant. The pH of all solutions was adjusted to 7.8 prior to addition to the Warburg flask.

Catalase. A modified procedure given by Appleman (1) was used. The main compartment of the Warburg flask received 2 ml of H_2O_2 which gave a final concentration of 10 mM. One ml of dilute enzyme preparation (supernatant diluted 10-fold) was added to the side arm. After equilibration in a 20°C water bath, the enzyme was tipped into the main compartment and oxygen evolution recorded at one minute intervals for 10 minutes.

Peroxidase. A manometric procedure similar to that described by Ettori (6) was used. The main compartment of the Warburg flask received 0.2 ml of 5 per cent pyrogallol, 1.5 ml of water, and 0.2 ml of 1 per cent hydrogen peroxide. A 0.2 ml aliquot of enzyme extract and 0.5 ml of 0.1 M potassium phosphate buffer were added to the side arm. After equilibration at 25°C the enzyme was tipped into the main compartment and gas evolution recorded. Carbon dioxide is evolved from pyrogallol reaction under the influence of peroxidase, and oxygen is evolved in the presence of catalase and hydrogen peroxide. A second set of duplicate flasks were included in which alkali and a small piece of folded filter paper were placed in the center well. These additional flasks gave an estimation of the oxygen evolved by catalase activity which had to be accounted for in the total gas evolution of the other flask.

RESULTS AND DISCUSSION

Gas exchange during the oxidation of lactic acid in supernatants is shown in Figure 1. The enzyme activity was equal to or greater than the ascorbic acid oxidase activity of the same tissue (4). The small amount of carbon dioxide evolved was thought to occur from the action of hydrogen peroxide on pyruvate forming acetate and water. The ratio of carbon dioxide evolved to oxygen consumed was very low, approximately 0.1 during lactate oxidation.

Glycolic acid oxidase of cotton tissue had a broad pH optimum for activity between 7.8 and 8.8 (Figure 1). Zelitch et al. (14) observed the same kind of pH curve for this enzyme in tobacco.

Glycolic acid oxidase has been shown to be inhibited by α-hydroxysulfonate compounds (11). The inhibition of this oxidase in supernatants of cotton tissue homogenates by two of these inhibitors is shown in Figure 2. Sodium acetaldehyde bisulfite and sodium bisulfite alone were equally effective in these studies. It is generally considered that bisulfite reacts with pyruvate which is produced in the oxidation of lactate forming pyruvic bisulfite as the inhibitor.

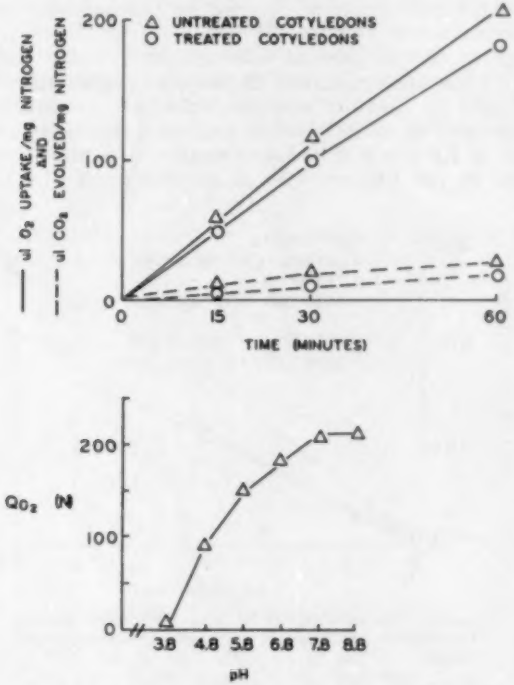

Figure 1. Gas exchange (upper) during oxidation of lactate in homogenate supernatants and the effect of pH (lower) on oxidation of lactate.

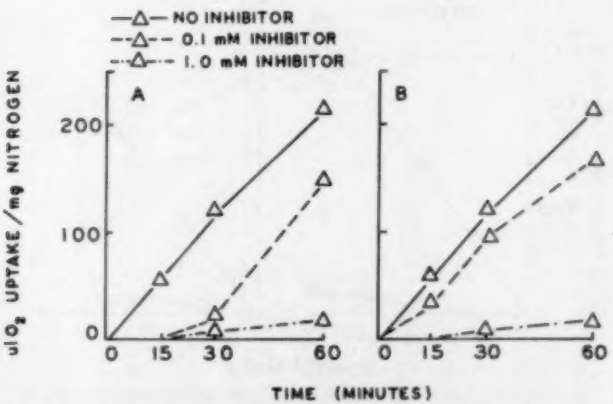

Figure 2. The inhibition of glycolic acid oxidase in homogenate supernatants by (A) sodium bisulfite and (B) acetaldehyde bisulfite. Lactate was used as substrate.

Through the inhibition of catalase by cyanide, the net oxygen uptake was increased (Figure 3).

When glycolate was used as substrate for glycolic acid oxidase, the QO_2 (N) was approximately 35 per cent higher than that with lactate (Figure 4). Bisulfite was also an effective inhibitor in this case. It appeared that the glycolic acid oxidase system could be a mechanism of hydrogen transfer to oxygen in cotton tissue.

As noted in the Figures 1 to 4, untreated and DCMA-treated

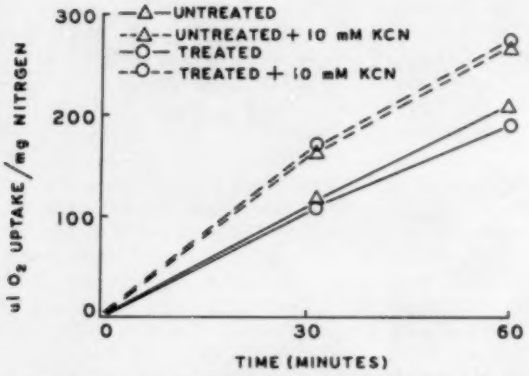

Figure 3. The influence of KCN on the oxygen uptake in homogenate supernatants during oxidation of lactate.

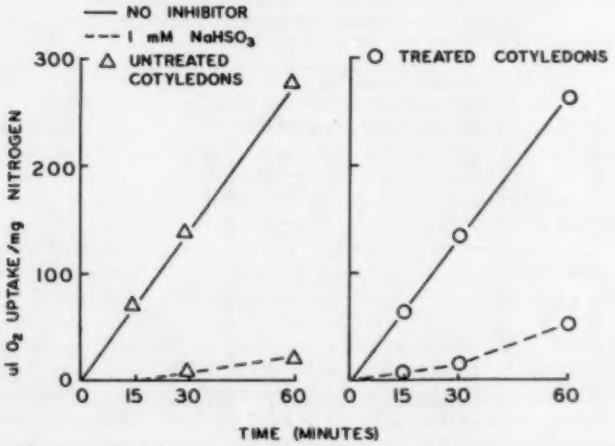

Figure 4. Oxidation of glycolate in homogenate supernatants of untreated and treated cotyledon tissue and inhibition by sodium bisulfite.

tissues were used and compared. DCMA had no apparent effect on the activity of glycolic acid oxidase.

The data discussed above support the hydrogen transfer system (11) involving the following reactions:

$$2\ \text{Glycolate} + 2\ O_2 \xrightarrow[\text{oxidase}]{\text{Glycolic acid}} 2\ \text{Glyoxylate} + 2\ H_2O_2$$

$$2\ H_2O_2 \xrightarrow{\text{Catalase}} 2\ H_2O + O_2$$

When cyanide was used for inhibition of catalase, net oxygen uptake should have theoretically been increased.

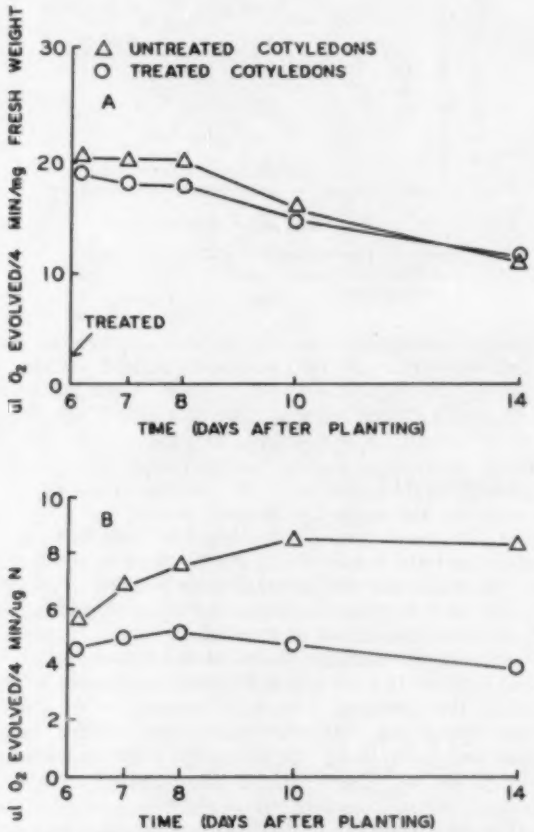

Figure 5. The influence of DCMA on catalase activity in supernatants (1000 × g × 7 minutes) of cotyledon homogenates based on (A) Fresh weight and (B) Nitrogen.

Catalase performs the highly specialized function of catalyzing the decomposition of hydrogen peroxide to water and oxygen. The studies with catalase following DCMA treatment of cotyledons and true leaves are reported in Figures 5 and 6. The treated tissue, four days and two days after treatment for cotyledons and true leaves, respectively, yielded approximately 50 per cent of the catalase activity as control tissue.

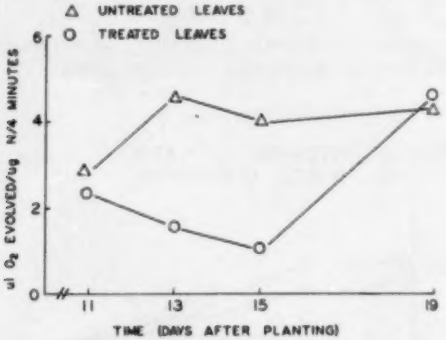

Figure 6. The influence of DCMA on catalase activity in supernatants of first true leaf homogenates.

In the case of cotyledons, catalase activity was held at the original level by DCMA, while in control tissue catalase activity increased two-fold during the eight-day testing period. Although supernatants were used, catalase activity was also placed on the basis of the original fresh weight. Surprisingly, enzyme activity on this basis was the same for treated and untreated tissue. Thus, through controlling growth DCMA also held the catalase content at the same level throughout the eight-day testing period.

Similar studies were conducted using first true leaf tissue. In this case, catalase activity was reduced for at least four days following treatment. As indicated for ascorbic acid oxidase (4), the effect of DCMA came earlier after treatment and recovery came earlier for true leaf tissue as compared to cotyledon tissue.

DCMA caused very similar effects on the activity of ascorbic acid oxidase and catalase in both cotyledon and leaf tissue. While catalase and ascorbic acid oxidase activity appeared to be the same for treated and untreated cotyledon tissue on a fresh weight basis, glycolic acid oxidase activity was about the same on a nitrogen basis.

Peroxidases are enzymes which in the presence of hydrogen peroxide catalyze the oxidation of many phenols and aromatic amines. It is possible that some of the hydrogen peroxide produced by way of glycolic acid oxidase is utilized through this channel and, thus, the oxygen evolved by catalase does not equal one-half that consumed.

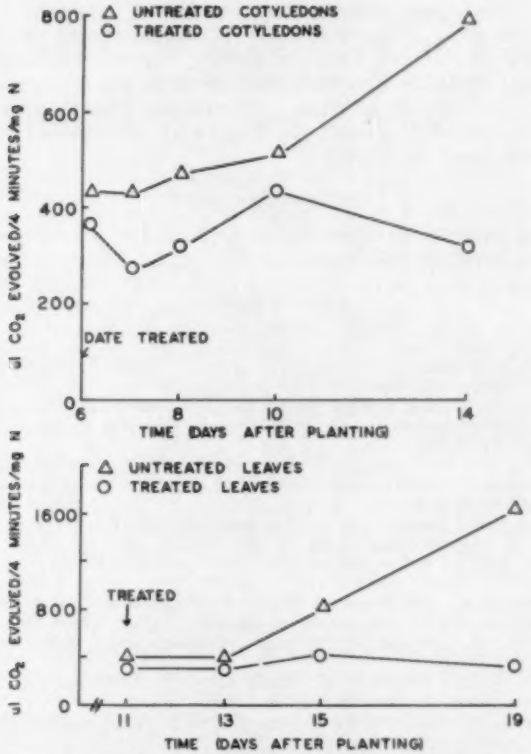

Figure 7. Effect of DCMA on peroxidase activity in supernatants of cotyledon homogenates (upper) and first true leaf homogenates (lower) at various intervals after treatment.

The activity of peroxidase was rather low in both cotyledon and leaf tissue until the second leaf began to expand (Figure 7). At this time, the activity of peroxidase began to rise and in six days was four times the original level. DCMA held the activity at the original level in both tissues during this period.

SUMMARY

A possible terminal oxidation system containing glycolic acid oxidase and catalase was investigated. The rate of oxidation of l-lactic and glycolic acids was equal to or greater than oxidation of ascorbate in cotton tissue homogenates. Inhibitors of glycolic acid oxidase also suggested that this enzyme was present. N–(3,4–dichlorophenyl)methacrylamide (referred to as DCMA) had no apparent effect on glycolic acid oxidase activity.

Catalase and peroxidase activity remained constant following DCMA treatment while untreated plants increased in levels of activity of these enzymes. Catalase activity, like ascorbic acid oxidase, was reduced by DCMA to the same extent as the effect on growth.

Unlike catalase and ascorbic acid oxidase, glycolic acid oxidase activity was about the same for untreated and treated cotyledon tissue when based on nitrogen.

ACKNOWLEDGMENT

Acknowledgment is made to Dr. J. B. Baker for helpful advice relating to experimental work.

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The Chemical Composition of Medusahead and Downy Brome[1]

RODNEY W. BOVEY[2], DUANE LE TOURNEAU, and LAMBERT C. ERICKSON[3]

INTRODUCTION

MEDUSAHEAD, *Elymus caput-medusae* L., an exotic winter annual grass, was introduced into northeastern Washington and probably adjacent Idaho over 50 years ago (6). Official records show its presence in southwestern Idaho 15 years ago, but ranchers in this area assert that it was present 25 to 30 years ago. Prior to 1950 it was known to infest a few thousand acres in southwestern Idaho and its distribution from 1945 to 1950 appeared rather static.

Since 1950, medusahead has become alarmingly aggressive. Estimates of the infested area in southwestern Idaho have increased from 20,000 acres in 1952[4] to 700,000 acres in 1958.[5] In a decade its position has changed from a minor problem to one of major concern to the range livestock industry. This problem is three-fold: first, it suppresses desirable vegetation due to its competitive ability; second, it is unpalatable to livestock at all stages of growth; and third, the dead vegetation decomposes slowly, thereby forming a persisting dense duff layer on the soil surface. In addition, as the plant matures it develops long barbed awns which cause mechanical injury to the eyes, noses, and mouths of grazing animals.

Medusahead is invading vast acreages formerly dominated by perennial grass species and more recently by downy bromegrass, *Bromus tectorum* L. The latter, although not a desirable range grass, provides considerable early spring forage. The growth habits, life cycles, and ecological adaptation of these two species appear to be similar. They typically grow in association until medusahead becames dominant and eventually exclusive.

No previous research has been reported on the composition of medusahead. Palatability differences of the two species stimulated studies on their comparative chemical composition and on methods to improve livestock utilization of medusahead. Such studies might reveal factors which could influence palatability and nutritive value. Data on the chemical composition of downy brome and many other grass species were previously available (4).

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[4]Anonymous. Noxious Weeds in the Columbia Basin. Noxious Weed Control Task Force. Columbia Basin Inter-Agency Committee. 1952.

[5]Fallon, Delbert, Bureau of Land Management, personal correspondence.

MATERIALS AND METHODS

Samples for these studies were obtained from an area in Nez Perce County in northern Idaho. The native grasses in this area had been replaced by medusahead and downy brome due to over-grazing and possibly persisting infestations of grasshoppers[6]. In this area, these grasses now exist in both mixed and adjacent pure stands.

To provide materials for these studies, respective pure stands were divided into grazing-protected and non-protected blocks. Replicated plots within each block were fertilized at 0, 20, and 160 pounds of nitrogen per acre in the fall, to provide sample areas the following year. The protected blocks provided materials for the chemical composition analyses while the unprotected medusahead block provided materials for grazing and silage studies.

Chemical composition samples were obtained from each of 3 replicated plots and combined for each nitrogen rate, at 5 harvest dates. The plants were severed at the soil surface. Thus, as the season advanced, the samples were composed of leaves; leaves and culms; and leaves, culms, and inflorescences. The sampling dates and stages of development for medusahead were: May 9—leaf, May 23—heading, June 6—flowering, June 21—late dough, and July 3—mature. Downy brome matures earlier and its development at these dates was: May 9—heading, May 23—flowering, June 6—late dough, June 21—mature, and July 3—mature.

Sub-samples for moisture determinations were placed in moisture tins and immediately brought to the laboratory where they were weighed, dried to constant weight at 100°C. and re-weighed. The remainder of each sample was autoclaved 5 minutes at 5 pounds pressure to inactivate enzymes. The material was then dried in a convection oven at 60°C. ground in a Wiley mill, and stored in sealed containers. Prior to analysis the ground material was further dried 48 hours in a vacuum oven. Duplicate determinations for crude fat, crude protein, crude fiber, lignin and ash were made according to official methods (1).

RESULTS AND DISCUSSION

The results of the chemical analyses of medusahead and downy brome obtained from the non-fertilized plots are given in Tables 1 and 2, respectively. The results for crude protein, crude fat, crude fiber, lignin, and ash are present on a dry-weight basis.

At comparable harvest dates, medusahead contained a higher percentage of moisture than downy brome. Typically downy brome matures earlier than medusahead. In this study downy brome preceded medusahead by at least one growth stage throughout the season. At comparable stages of development, the two species were approximately equal in moisture content. As expected, moisture content decreased as the plants matured.

When collected at the same date, medusahead contained more

[6]Portman, Roland, Correspondence and Grasshopper Survey Reports. Department of Entomology, University of Idaho.

Table 1. The percentage composition of medusahead, *Elymus caput-medusae* L., at five stages of development. All percentages other than moisture are expressed on a dry weight basis.

	Stage of growth				
	Leaf 5-9-58	Heading 5-23-58	Flowering 6-6-58	Dough 6-21-58	Mature 7-3-58
Moisture	66.8	62.7	50.3	34.9	—
Protein	10.4	8.8	6.8	6.5	7.3
Fat	2.6	2.3	1.8	1.6	1.0
Crude fiber	26.8	27.4	31.2	28.1	30.5
Lignin	6.1	9.7	8.6	10.0	7.9
Ash	13.9	13.7	12.8	14.7	14.5

Table 2. The percentage composition of downy brome, *Bromus tectorum* L., at five stages of development. All percentages other than moisture are expressed on a dry weight basis.

	Stage of growth				
	Heading 5-9-58	Flowering 5-23-58	Late dough 6-6-58	Mature	
				Early 6-21-58	Late 7-3-58
Moisture	64.6	49.0	30.3	8.2	—
Protein	8.0	8.8	7.1	4.6	4.7
Fat	1.5	2.0	1.8	1.3	1.2
Fiber	31.3	24.5	27.2	35.6	39.6
Lignin	8.4	8.5	9.7	11.2	11.4
Ash	8.1	8.7	8.7	9.9	8.0

than or as much crude protein as downy brome. When compared at equivalent stages of development, however, the two species are relatively comparable in protein content. Generally, the protein content of both species decreased as the plants matured. The protein content of medusahead is comparable to that of many other grass species (4). In the absence of data on protein quality, it appears that medusahead would be comparable in nutritive value to many desirable grass species.

At the same harvest dates medusahead contained more crude fat and generally less lignin than downy brome. The crude fiber content, althongh variable, was higher in downy brome as the plants reached maturity. Both species decreased in crude fat content and increased in lignin as the plants matured. The percentages of crude fiber and lignin for medusahead are not high when compared to downy brome or to many desirable forage grasses.

The ash of medusahead was found to comprise 13 to 15 percent of the dry matter. This was significantly higher than the ash content of downy brome. This amount of ash is also greater than that reported for many other grasses or forages. It was of interest, therefore, to characterize more fully the components of the ash of medusahead. Samples of medusahead and downy brome collected in 1957 and prepared in the same manner as previously outlined were used

Table 3. The ash and mineral constituents of medusahead and downy brome collected May 8, 1957. All results are reported on the basis of the dry weight of the whole plant.

	Medusahead	Downy brome
Total ash	15.5%	9.6%
Phosphorus	0.22	—
Calcium	0.66	—
Magnesium	0.16	—
Potassium	1.20	—
Water insoluble ash	14.0	6.5
Silica	11.3	4.4
Alkalinity of ash	44.7[a]	46.9
Alkalinity of soluble ash	7.4	18.9
Alkalinity of insoluble ash	34.6	27.5

[a]Alkalinity of ash is expressed as the number of ml. of 1N HCL to neutralize the ash of 100 gms. of sample.

in this portion of the study. The results of these analyses, expressed on a dry weight basis, are given in Table 3.

The ash content of the 1957 samples of downy brome and medusahead were 9.6 and 15.5 per cent, respectively. Analyses for phosphorus (2), magnesium and calcium (7), and a flame photometric determination of potassium showed these elements made up a relatively small portion of the ash of medusahead. Alkalinity of total ash determinations (3) showed little difference between the two species. However, when ash alkalinity was determined, most of the ash of medusahead was found to be insoluble in dilute HCl. Fractionation of the total ash into water soluble and non-soluble components (3) showed that about 90 per cent of the ash of medusahead was insoluble in water. Microscopic examination of the ash showed what appeared to be silicified cell wall material. Determination of the silica content (5) revealed that over 70 per cent of the ash or about 11 per cent of the dry matter of medusahead was silica. Comparable figures for downy brome were 47 and 4.4 percent. This high silica content of medusahead may contribute to its resistance to decomposition.

Similar chemical analyses were conducted on vegetation from the nitrogen fertilized plots. Because of the similarity of the results, these data are omitted. Briefly, for both species; the nitrogen treatments produced significant (at the 5 per cent level) increases in moisture and protein contents at all collection dates, while the differences in crude fat, crude fiber, and lignin were non-significant.

The influence of nitrogen application on forage utilization was determined by giving free access to grazing cattle and horses. They consumed 90 per cent of the medusahead on the 160-pound nitrogen plots, 40 per cent on the 20-pound nitrogen plots, and a non-discernible quantity on the check plots. Nitrogen fertilization increased utilization, but even at the excessive rate of 160 pounds per acre, 10 per cent of the plants developed viable seed. Thus the nitrogen treatments resulted in some medusahead control but did not produce benefits commensurate with the cost.

Samples for silage were collected from the non-protected check plots on May 23, June 6, 13, and 20. These samples were conditioned with the following silage additives: none, molasses, beet pulp, and molasses plus beet pulp. After 4 months the samples were placed in a pen containing 40 hungry sheep that were accustomed to a ration containing silage. All the samples were rejected. Therefore, to date, there appears to be no satisfactory method whereby medusahead can be utilized by animals. This rejection or avoidance by animals may be influenced by the high silica content or there may be other undetermined factors involved.

SUMMARY

The moisture, crude protein, crude fat, crude fiber, and lignin contents of medusahead (*Elymus caput-medusae*) were comparable to that of downy brome and many desirable grass species. The ash content of medusahead, however, was found to be much greater than that of downy brome and of many other grasses. The ash of medusahead contained silica amounting to over 10 per cent of the dry weight of the plant. The high silica content of medusahead is the basis for its harshness and may partially explain its unattractiveness to livestock. The high silica content may also be the reason why the plant is slow to decompose in the field. Nitrogen fertilization increased forage consumption by cattle and horses. Medusahead silage, whether pure or modified with additives, was unacceptable to sheep.

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The Herbicidal Effect of EPTC, CDEC, and CDAA on *Echinochloa crusgalli* with Various Depths of Soil Incorporation[1]

FLOYD M. ASHTON and KENNETH DUNSTER[2]

THE use of pre-emergence herbicides where natural rainfall is low and/or sporadic has not been uniformly successful. In general, the results have been erratic; excellent weed control has been obtained sometimes and a complete lack of weed control at other times. Good weed control is usually obtained when one-half inch or more of rain falls within a few days after application. This result is confirmed in irrigated regions by the fact that overhead irrigation usually gives uniformly good weed control. The standard furrow irrigation usually gives erratic results; however, a furrow irrigation which thoroughly saturates the soil with water to the surface may give good results. Since this type of furrow irrigation is difficult to achieve in practice and the equipment necessary for overhead irrigation is not widely used, the physical incorporation of the herbicides in the top few inches of soil is being widely field tested as a method of application in these areas of low and sporadic rainfall. In general, such incorporation has resulted in a much improved performance under furrow irrigation in the absence of rainfall (1, 2, 3, 4).

The experiments reported here were conducted under controlled conditions to establish certain principles which might be an aid in the applied aspects of the incorporation of pre-emergence herbicides into the soil. The experiments were designed to show whether increasing depths of incorporation could reduce the effectiveness of herbicides by excessive dilution with the soil.

METHODS

The methods developed and described here had to meet the following requirements: A given quantity of herbicide had to be incorporated to a given depth; it had to remain in this position through the experiment; a test species had to be grown to test the herbicidal effectiveness of the various treatments. Since all herbicides move to some extent wtih water movement in soil, neither watering the plants by addition of water to the soil surface or placing the tin cans in a tray of water could be used. Yet, sufficient water was required to raise a test species. This was accomplished by bringing the soil to field capacity and covering the tin cans with polyethylene bags.

In all treatments except the surface application air dry soil was added to number two tin cans ½ inch at a time. After the addition

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of each half-inch increment of soil distilled water or distilled water plus the herbicide was carefully added to give uniform wetting of the previously added ½ inch of soil. The amount of water which was added was just sufficient to bring that ½ inch of soil to field capacity. Twenty-five seeds of *Echinochloa crusgalli*, barnyardgrass, were placed on the soil surface just prior to the addition of the final half-inch increment of soil. In the surface application, 2½ inches of air dry soil were added to the cans, 25 barnyardgrass seeds were added to the soil surface, one-half inch of soil was added to the can, and the entire soil mass brought to field capacity with distilled water. The herbicide was then carefully sprayed on the surface with an atomizer. Three bamboo pot stakes were placed in the soil at uniform distances at the periphery of each can and extended six inches above the soil surface. A polyethylene bag was placed over the stakes and tightly sealed to the outside of the tin can with a rubber band.

The soil was Yolo sandy loam which had been screened through a one-eighth inch mesh screen, air dried, and steam sterilized. The field capacity of this soil was 17.0 per cent. The herbicides used were commercial formulations of ethyl *N*,*N*–di–*n*–propylthiolcarbamate (EPTC), 2–chloroallyl diethyldithiocarbamate (CDEC), and 2–chloro–*N*,*N*–diallylacetamide (CDAA). The herbicides were applied at 1, 2, and 4 pounds per surface acre. CDAA was also applied at ¼ and ½ lb/A; these lower rates were required because CDAA was more toxic to barnyardgrass than EPTC or CDEC under these conditions. The herbicides were applied to the surface or incorporated to depths of ½, 1, 2, and 3 inches. The number of plants were counted and fresh weights taken 14 days after planting. Each treatment was replicated three times. The data are presented as percentages of the untreated control, based on mean fresh weight per plant. The data were analyzed statistically by a three by five factorial analysis for EPTC and CDEC, and five by five factorial analysis for CDAA.

RESULTS AND DISCUSSION

Each of the three herbicides performed somewhat differently, therefore the results and discussion of each are presented separately. A discussion of certain common characteristics follows the individual herbicide discussion.

EPTC. The data are presented in Figure 1. The analysis showed a significant difference between the three rates of application as well as the five depths of incorporation. In all treatments barnyardgrass emerged from the soil, but in the most effective treatments the seedlings were stunted with a knotty malformation and a weight reduction. The incorporation of EPTC to a depth of 1 inch when the weed seed was at ½ inch depth appeared to be the most effective position. The incorporation of EPTC to greater depths reduced its effectiveness because of the dilution in a greater volume of soil. The effect of the depth of incorporation was not as apparent at

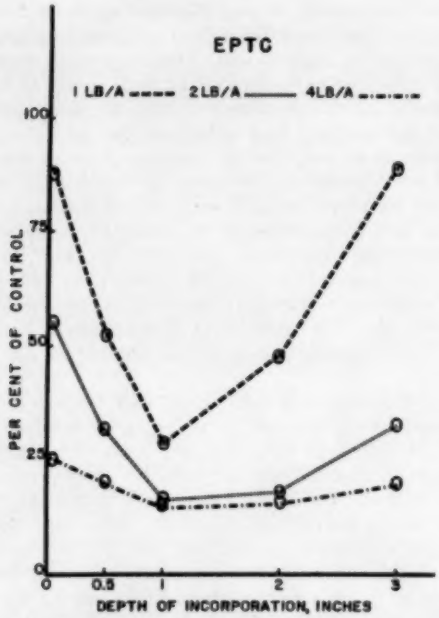

Figure 1. The effect of various depths of soil incorporation on the effectiveness of EPTC in reducing the yield of barnyardgrass. The data are presented as a percentage of the untreated control based on the mean fresh weight per plant.

4 lb/A as at the lower concentrations. This was because the concentration of herbicide near the germinating seed was sufficient at all depths of incorporation to almost completely inhibit growth.

CDEC. The data are presented in Figure 2. The analysis showed a significant difference between three rates of application as well as the five depths of incorporation. As with EPTC, the affected barnyardgrass seedlings emerged from the soil but were stunted with a knotty malformation and weight reduction. The growth of barnyardgrass was almost equally inhibited by 1, 2, and 4 lb/A of CDEC applied to the surface of the soil or incorporated to a depth of one-half inch. This was also true for 2 and 4 lb/A at the one-inch incorporation depth, however, at 1 lb/A the dilution effect was apparent at the one-inch depth of incorporation. The dilution effect was also apparent at 1 and 2 lb/A at the two- and three-inch depths of incorporation. The herbicide markedly inhibited growth at all depths of incorporation at 4 lb/A, therefore the effect of the depth of incorporation was not observed.

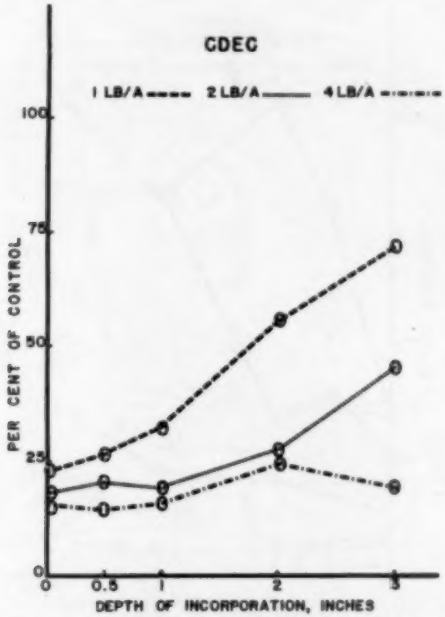

Figure 2. The effect of various depths of soil incorporation on the effectiveness of CDEC in reducing the yield of barnyardgrass. The data are presented as a percentage of the untreated control based on the mean fresh weight per plant.

CDAA. The data are presented in Figure 3. The analysis showed a significant difference between the five rates of application and the five depths of incorporation. As with EPTC and CDEC, the affected barnyardgrass seedlings emerged from the soil but were stunted with knotty malformations and weight reduction. The growth of barnyardgrass was almost equally inhibited at all depths of incorporation at 2 and 4 lb/A. The results from 1 lb/A showed slightly less toxicity but the dilution effect was not apparent except at the three-inch depth of incorporation. The dilution effect was quite apparent at ¼ and ½ lb/A.

In Figure 4, the data for all three herbicides at the one, two, and three inch depths of incorporation are presented as concentration values, pounds per acre-inch, rather than as the surface rate of pounds per acre. The data showed that the depth of incorporation was a critical factor in evaluating the herbicidal effectiveness of these herbicides. The deeper the incorporation or the lower the concentration of the herbicide in the soil the less effective the herbicide. Under the conditions of these experiments (Figure 1)

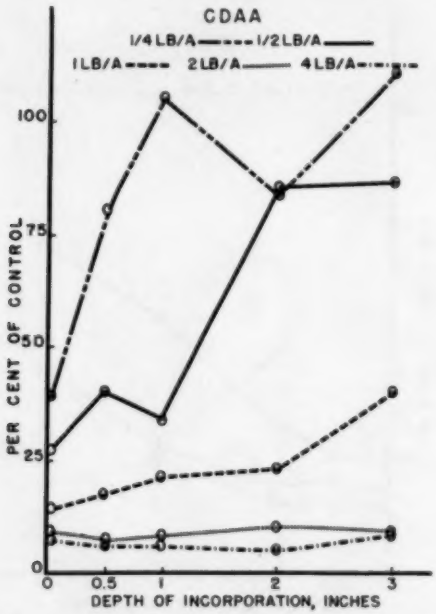

Figure 3. The effect of various depths of soil incorporation on the effectiveness of CDAA in reducing the yield of barnyardgrass. The data are presented as a percentage of the untreated control based on the mean fresh weight per plant.

1 lb/A of EPTC incorporated to a depth of one inch (one pound per acre-inch) was an effective herbicide for barnyardgrass but 1 lb/A of EPTC incorporated to a depth of three inches (0.33 pounds per acre-inch) was not effective. In each case the rate of application based on surface area was the same but the herbicidal effectiveness was not the same; when the third dimension, depth, is taken into consideration the rate of application reflects the herbicidal effectiveness.

It is therefore proposed that the rate of application of soil-incorporated herbicides include the depth factor and be expressed as pounds per acre-inch rather than the accepted unit of pounds per acre which does not take into account this critical factor of depth.

SUMMARY

The effectiveness of EPTC, CDEC, and CDAA was markedly influenced by the depth of soil incorporation. The herbicides became less effective as they were incorporated deeper into the soil because

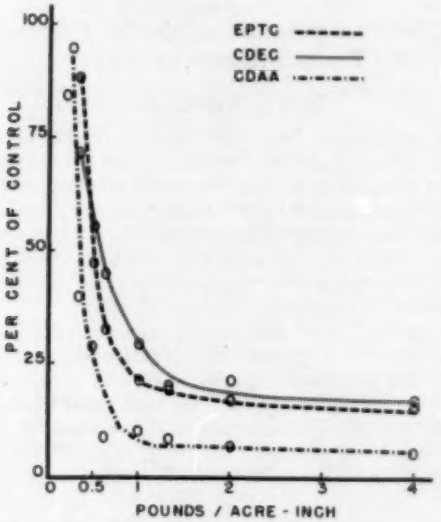

Figure 4. The herbicidal effectiveness of EPTC, CDEC, and CDAA expressed as pounds/acre-inch.

the soil acted as a diluting medium, lowering the actual concentration of the herbicide in the vicinity of the germinating weed seed.

It is proposed that the rate of application of soil incorporated herbicides be expressed as pounds per acre-inch rather than pounds per acre. The proposed unit takes into account the critical factor of the extent of the depth of incorporation as a dilution factor.

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3,4–Dichloropropionanilide for Control of Barnyardgrass in Rice[1]

ROY J. SMITH, JR.[2]

IN Arkansas isopropyl *N*–(3–chlorophenyl)carbamate (CIPC) is recommended for controlling barnyardgrass (*Echinochloa crusgalli* (L.) Beauv.) in rice. Method and depth of seeding, time of application, and water management after treatment are critical when using CIPC to control barnyardgrass in rice (2,3,4). Rice is very susceptible to CIPC when roots of the seedlings contact the herbicide (1,4). A herbicide that is more selective and less sensitive to management and environment is needed to replace CIPC.

In greenhouse and field studies 3,4–dichloropropionanilide[3] was evaluated in 1959 for controlling barnyardgrass in rice. The objectives of the investigations were: (1) to determine the effect of 3,4–dichloropropionanilide on rice and barnyardgrass as influenced by rate and time of application, volume of solution, and method and depth of seeding, and (2) to study the response of different varieties of rice to 3,4–dichloropropionanilide.

METHODS AND RESULTS

Greenhouse experiments.

Several experiments were conducted by filling metal flats (10 × 14 × 3½ inches) and/or metal pots (8 × 6 inches) with Crowley silt loam and seeding them to barnyardgrass and/or rice. Seeds of barnyardgrass were incorporated throughout the mass of soil in each pot or flat and Nato or Bluebonnet 50 varieties of rice were seeded by water planting or by dry seeding at depths of ½ to 1½ inches. The herbicide 3,4–dichloropropionanilide was applied at rates of 2, 4, 8, 12, 16, and 32 lb/A (active herbicide) in water solutions of 5, 10, 20, and 40 gallons per acre. Three replicates arranged as a randomized complete-block design were used in the investigations.

In one greenhouse study, rice was grown by placing Nato seed in petri dishes and adding 3,4–dichloropropionanilide at concentrations of 0.5, 1, 2, 4, 8, 16, 32, 64, 128, 256, 512, and 1024 ppm. The growth of the primary roots of the rice seedlings was expressed as elongation in percent of the untreated control.

At rates which controlled barnyardgrass, method and depth of seeding, variety of rice, and volume of application did not affect greatly the response of rice to 3,4–dichloropropionanilide. At rates

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[3]The author wishes to acknowledge the cooperation of the Rohm & Haas Company, Philadelphia, Pennsylvania in supplying the compound for these investigations.

Table 1. Effect of 3,4–dichloropropionanilide on rice grown in the greenhouse as influenced by variety, method and depth of seeding, and rate of treatment.

Variety, method and depth of seeding	Rating[a] when applied at, lb/A					
	0	2	4	8	16	32
Nato:						
Water seeding	0	0	0	1.3	1.7	6.0
Dry seeding—½ inch	0	2	0	0	1.3	3.7
Dry seeding—1½ inches	0	0	0	0	1.3	2.7
Bluebonnet 50:						
Water seeding	0	0	0	0	2.3	3.7
Dry seeding—½ inch	0	0	0	0.7	1.0	4.3
Dry seeding—1½ inches	0	0	0	0	0	1.3

[a]Values are injury ratings: 0, no injury; 1–3, slight injury; 4–6, moderate injury; 7–9, severe injury; 10, all plants killed.

of 2, 4, 8, and 16 lb/A varieties Nato and Bluebonnet 50 treated in the first- to second-leaf stages of growth were only slightly injured by 3,4–dichloropropionanilide regardless of method or depth of seeding, but a rate of 32 lb/A injured the rice (Table 1).

When 3,4–dichloropropionanilide was applied at 2 lb/A, a volume of 5 gallons per acre caused significantly better control of barnyardgrass than 10, 20, or 40 gallons per acre (Table 2). The herbicide

Table 2. Effect of volume and rate of application of 3,4–dichloropropionanilide on percent control of barnyardgrass grown in the greenhouse.

Rate of herbicide lb/A	Per cent control[a] of barnyardgrass				
	Gallons per acre applied				Mean
	5	10	20	40	
2	73	63	60	60	64
4	73	63	70	83	72
8	83	83	87	80	83
Mean	76	70	72	74	

[a]LSD for rate means: 5%, 5; 1%, 6.
LSD for rate x volume means: 5%, 9; 1%, 13.

applied at 4 lb/A in 40 gallons per acre gave best control of barnyardgrass. When this herbicide was applied at 8 lb/A the gallonage did not affect the activity of the herbicide on barnyardgrass. As the rate of application of 3,4–dichloropropionanilide increased control of barnyardgrass increased. The herbicide applied at the rates and volumes depicted in Table 2 did not injure the rice.

Time and rate of applying 3,4–dichloropropionanilide were important variables. Barnyardgrass was not controlled when 3,4–dichloropropionanilide was applied before the grass emerged (Table 3). When applied at 2 lb/A to barnyardgrass that was in the second-leaf stage, 70 percent control was obtained but 2 lb/A applied at other stages did not control barnyardgrass adequately. Applications in the first-leaf stage did not control the grass adequately. Applications at rates of 4, 8, and 12 lb/A in the second-, third-, and fourth-leaf stages gave 87 percent or better control of barnyardgrass. Best

Table 3. Effect of time and rate of application of 3,4–dichloropropionanilide on percent control of barnyardgrass grown in the greenhouse.

Stage and height of grass at time of spraying	Per cent control of barnyardgrass				
	Rate of herbicide—lb/A				Mean
	2	4	8	12	
	Experiment 1[a]				
Immediately after seeding	0	0			0
Just before emergence	0	0			0
First-leaf (2 in.)	20	47			33
Second-leaf (3 in.)	70	100			85
Third-leaf (5 in.)	7	87			47
	Experiment 2[b]				
First-leaf (1 in.)	27	63	73	87	62
Second-leaf (2 in.)	70	87	90	93	85
Third-leaf (5 in.)	53	87	90	90	80
Fourth-leaf (10 in.)	60	87	90	90	82

[a]LSD for stage means: 5%, 6; 1%, 9.
LSD for stage x rate means: 5%, 9; 1%, 12.
[b]LSD for stage means: 5%, 6; 1%, 8.
LSD for stage x rate means: 5%, 11; 1%, 15.

control of barnyardgrass was obtained by applying 3,4–dichloropropionanilide when the largest grass reached the second- to third-leaf stages of growth (2 to 5 inches tall) and 4 lb/A gave adequate control.

Rice treated in the third- to fourth-leaf stages of growth with 8 and 12 lb/A of 3,4–dichloropropionanilide was injured as manifested by chlorosis and death of seedings. At 4 lb/A there was a mottled chlorosis on the leaves but the seedlings outgrew the injury soon after treatment.

Root elongation of rice that was grown in solutions of 3,4–dichloropropionanilide was inhibited about 25 percent at concentrations of 0.5, 1, 2, 4, 8, 16, and 32 ppm. Root elongation was inhibited 53, 97, 96, 98, and 99 percent at herbicide concentrations of 64, 128, 256, 512, and 1024 ppm, respectively.

Field experiments.

In two experiments the Zenith variety of rice was drilled at a depth of approximately 1½ inches at 120 to 125 lb/A during May and June 1959. In one experiment, 3,4–dichloropropionanilide at 1, 2, and 4 lb/A was sprayed early post-emergence when rice and barnyardgrass were in the first-leaf stage of growth and in the other test the herbicide was sprayed 1-week pre-planting, immediately pre-planting, and pre-emergence at rates of 3 and 6 lb/A. In the pre-planting treatments the herbicide was incorporated into the surface 1 or 2 inches of soil by section-harrowing lightly after spraying. The experiments were replicated and arranged as randomized complete-block or split-plot designs. Fertilization of the plots was based on soil test.

3,4–Dichloropropionanilide at 3 and 6 lb/A applied either pre-planting or pre-emergence did not control barnyardgrass or injure rice. The herbicide applied post-emergence at 1, 2, and 4 lb/A gave

Figure 1. Control of barnyardgrass in rice with 3,4–dichloropropionanilide. Plots sprayed early post-emergence with 4 lb/A of 3,4–dichloropropionanilide (1) and unsprayed (2).

75, 80, and 90 percent control of barnyardgrass, respectively, and yields of 56, 90, and 88 bu/A, respectively, as compared to an average yield of 18 bu/A on the untreated plots. No visible injury was evidenced on the treated rice. Figure 1 compares an untreated plot with a plot treated with 4 lb/A of 3,4–dichloropropionanilide applied early post-emergence.

DISCUSSION

Although there is no definite knowledge as to the mode of action of 3,4–dichloropropionanilide in plants, its mechanism of action appears to be different from that of CIPC. Since the activity of 3,4–dichloropropionanilide on barnyardgrass was low when applied as pre-planting and pre-emergence treatments and activity was high when the chemical was applied post-emergence, the herbicide probably enters the grass plant through the foliage rather than through the roots as is the case with CIPC. When 3,4–dichloropropionanilide is applied at rates of 2 and 4 lb/A, several days elapse between the time of spraying and the development of severe injury to the barnyardgrass, which may indicate that it is translocated to some degree within the seedling.

Results indicate that rice is tolerant to 3,4–dichloropropionanilide. Since rice treated in the first- to second-leaf stages of growth with 2, 4, 8, and 16 lb/A of the herbicide was injured only slightly, the margin of safety on rice would appear quite large. The safety margin of CIPC is narrow since 6 lb/A is required for control of barnyardgrass and 10 or 12 lb/A or less may cause severe injury to rice (4). Thus 3,4–dichloropropionanilide may have advantages in tolerance and safety over CIPC.

Since such factors as method and depth of seeding, time of application of herbicide, and water management after treatment appear to be less critical with 3,4–dichloropropionanilide than with CIPC (4), 3,4–dichloropropionanilide appears to be a much more versatile herbicide than CIPC and it may control barnyardgrass without injuring rice under many conditions where CIPC could not be used.

SUMMARY

Greenhouse and field investigations were made in 1959 to evaluate the effectiveness of 3,4–dichloropropionanilide for controlling barnyardgrass in rice. The important findings were:

1. Best control of barnyardgrass was obtained by applying 3,4–dichloropropionanilide in the first- to third-leaf stages of growth (2 to 5 inches high). When applied pre-planting or pre-emergence 3,4–dichloropropionanilide did not control barnyardgrass adequately.

2. A rate of 4 lb/A of 3,4–dichloropropionanilide gave adequate control of barnyardgrass but 1 or 2 lb/A did not. In the greenhouse, rice in the third- to fourth-leaf stages of growth was injured by this herbicide at 8 lb/A, but in the first- and second-leaf stages, 16 lb/A did not cause severe injury. Roots of rice grown in solutions of this herbicide were inhibited about 25 percent with concentrations of 0.5 to 32 ppm and severely in solutions from 64 to 1024 ppm.

3. In greenhouse experiments, the varieties Nato and Bluebonnet 50 responded similarly to 3,4–dichloropropionanilide, the method and depth of seeding rice did not affect the activity of the herbicide on rice, and the gallonage in which it was applied did not greatly affect control of barnyardgrass or injury to rice.

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BRIEF PAPERS

A Small Plot Sprayer Using Disposable Spray Containers[1]

D. H. Webster and J. S. Leefe[2]

A small portable sprayer was found to be the most practical for applying experimental herbicides for the control of lambkill (*Kalmia angustifolia* L.) in lowbush blueberry plots. The standard knapsack sprayer requires a large volume of rinse water to clean the sprayer between treatments and this frequently has to be transported a considerable distance to the spray site. In order to reduce the amount of rinse water to a minimum a knapsack sprayer[3] was modified so that the spray solution for each treatment could be held in an individual disposable polyethylene bag.

The outlet pipe on the tank of the sprayer was removed and replaced with a tire valve stem (Figure 1) (J); and the pump cylinder cut off 1¼ inch from the head and plugged with a rubber stopper; the head (H) was drilled and fitted with a 10 × ¼-inch copper tube (F) on the inside and the outlet hose (I) on the outside.

The upper disc (E) and rubber cup (D) of a 2-inch vacuum bottle stopper[4] were drilled to fit the copper tube. The aluminum disc, (C) on the stopper was replaced with one of sheet iron which was soldered to the copper tube 5½ inches below the head to give a rigid, airtight connection. A piece of Tygon tubing (B) 4 × ¼ inch was fitted over the end of the copper tube and a rubber stopper (A), with a channeled lower surface, fitted to the Tygon tube. An iron ring (G) of 2⅛-inch diameter was made to fit over the vacuum bottle stopper to hold the polyethylene bag in place.

In filling the sprayer the iron ring (G) is slipped over the neck of a polyethylene bag containing the spray solution and the position of the bag adjusted so that the rubber stopper (A) just reaches the bottom of the bag. The ring is then pulled over the rubber cup (D) and the stopper sprung to clamp the neck of the bag between the rubber cup and ring. The head (H) is then fitted to the spray tank and the system pressurized. A tire pump was used for this purpose, although any convenient source of pressure, e.g. a flask of CO_2, would be satisfactory.

The polyethylene bags can be filled quite easily by two persons working together; in this case one can hold the bag while the other pours in the spray solution. When the operator is working alone the most convenient method is to place the bag inside a clyindrical container, folding the edge of the bag over the top edge of the con-

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[2]Botanist, Nova Scotia Research Foundation, and Agronomist, Research Station, Kentville, Nova Scotia.

[3]Chapin No. 180, R. E. Chapin Mfg. Wks. Inc., Batavia, N. Y.

[4]"Snap-Tite Vacuum Bottle Stopper", Moeller Mfg. Co. (Canada) Ltd., Niagara Falls, Ontario.

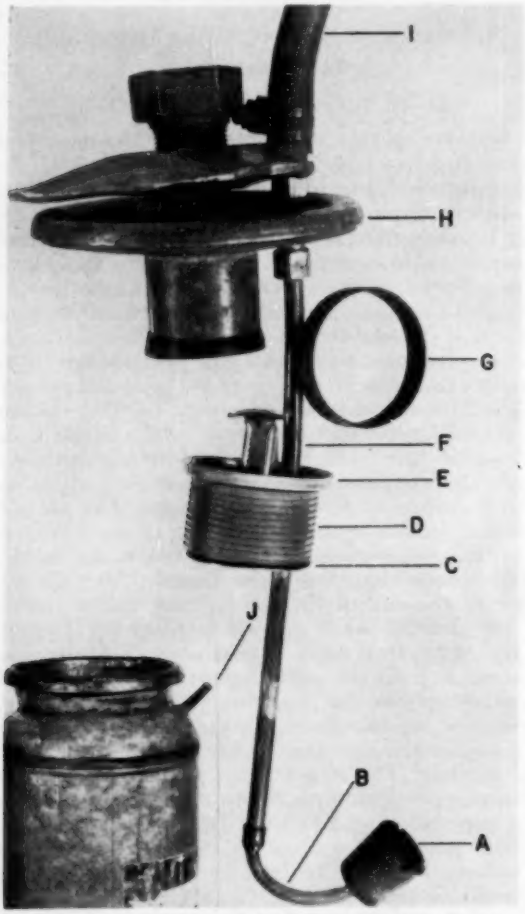

Figure 1. Parts of knapsack sprayer modified to take polyethylene bags.

tainer. The spray solution can then be easily poured in and the bag removed from the container and placed on the sprayer.

The polyethylene bag collapses as the spray solution is discharged. On completion of spraying the bag can be discarded and the parts exposed to the solution rinsed.

Polyethylene bags of 1.25 mil thickness and 3–5 pound capacity, depending on the volume of spray required, were found to be suitable. No difficulty with the bag breakage or leakage was encountered in the treatment of more than 300 plots with this sprayer.

A Variable Dosage Sprayer for Treating Small Plots[1]

J. S. Leefe

The principle of the variable dosage or logarithmic sprayer was described first in 1955 (3). Since that time at least two sprayers utilizing this principle have appeared (1, 2). Certain limitations were, however, encountered in adapting these sprayers for small plot testing under field conditions.

The Chesterford sprayer (1) is too large for use on small plots, and the Leasure sprayer (2), while designed for use on small plots, is powered by electric motors and consequently depends on a supply of electric power, which may not be readily available under average operating conditions.

A requirement for a small sprayer that would permit the rapid estimation of the approximate rate at which a herbicide could be expected to control a given weed species led to the development of the sprayer described here. The sprayer was patterned on the Leasure sprayer (2), but was mounted on a small 2 hp garden tractor, the engine of which propels the sprayer and drives the circulating pump for mixing in the concentrate tank. The spraying system is pressurized with compressed air which regulates the discharge from the nozzles.

The line diagram (Figure 1) shows the layout of the spraying apparatus and the photograph (Figure 2) illustrates the sprayer mounted on the tractor.

A source of compressed air or gas is required to pressurize the spraying system. A small, portable gasoline engine driven compressor with a storage tank of 4 cu ft at 120 psi capacity proved adequate for this purpose.

The solenoid valves[2] were operated with a 6 volt dry battery (i) (Figure 1). The concentrate tank (k) was made from a piece of 2½ inch brass tubing 10½ inches long. The pump[3] is a belt driven ¾ inch centrifugal type with a bronze impeller, circulating 9 gpm with an engine speed sufficient to propel the tractor at 100 fpm. The volume of the concentrate system, i.e. concentrate tank, pump and connecting pipes is 1470 cc.

The boom (q) was made from ⅜ inch stainless steel pipe using nozzles[4] with ball check valves, that open at 5 psi, in the strainer assembly.

The concentrate tank (k) was fitted with a tire valve stem (b) allowing the whole system, forward of the diluent tank (f), to be cleared with compressed air between runs.

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[1]Received for publication June 24, 1960. Contribution No. 1043 from the Research Station, Canada Department of Agriculture, Kentville, Nova Scotia.

[2]No. V5D2500–6 volt solenoid valve from Electric Valve Division, Skinner Chuck Co., New Britain, Conn.

[3]Centrifugal pump, ball bearing, ¾ inch end suction, open impeller in bronze fitted construction from Fairbanks Morse Co., Chicago, Ill.

[4]TeeJet No. 6502 from Spraying Systems Co., Bellwood, Ill.

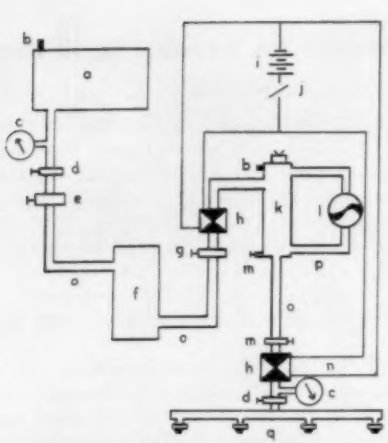

Figure 1. Line diagram showing the layout of the variable dosage sprayer.

Figure 2. The variable dosage sprayer mounted on garden tractor.

The tractor was fitted with a speedometer[5] which was calibrated on the sprayer over 100 ft runs against time. Speedometer readings equivalent to various ground speeds were recorded for use during spraying operations.

[5]No. 366–AU Farm Speedometer from Stewart Warner Corp., Chicago, Ill.

To operate the sprayer, the diluent tank (f) is filled and the reserve air tank (a) is charged with compressed air to 100 psi, the pressure regulating valve (e) having been adjusted previously to give the required pressure at the spray boom. The drain cock (g) and the gate valve (d) are opened and when diluent appears, the drain cock is closed bringing diluent to the first solenoid valve (h). The concentrate tank is filled with the drain cocks (m) and the drain in the bottom of the circulating pump open. When concentrate runs out, the drains are closed and the concentrate tank filled and closed. The engine is started and the sprayer driven to one end of the plot. The switch (j) is turned on in time to begin spraying as the boom passes over the beginning of the plot. With a little experience an operator can time the "switch on" very accurately.

The rate-time relationship for this system is given by Leasure (1):

$$\ln \frac{C_o}{C_t} = \frac{(kR-m)t}{V}$$

where C_o = original concentration
C_t = concentration at the end of time t
R = rate of flow
V = volume of the concentrate tank
k, m = constants for the particular system

On the assumption that the values of the constants would be unity or nearly so theoretical curves were calculated using the relationship:

$$\ln C_t = \ln C_o - \frac{Rt}{V}$$

The machine was then calibrated against time and over the ground, collecting samples at 10 ft intervals. Calcium chloride solution was used for the calibration, the concentration of $CaCl_2$ in the samples being determined with a refractometer. Numerous trials indicated that the constants k and m could, for all practical purposes, be taken as unity.

The results of two calibrations are given in Figure 3. The volume of the concentrate system is 1470 cc. It will be seen that with the tips used, the 50% concentration point occurs at approximately 40 ft. These tips apply 56 gpa at the sprayer speed (100 fpm) used here. Since the concentration at the end of any given time depends on the three variables, original concentration, rate of flow through the sprayer and the volume of the concentrate tank system, many combinations are possible. Leasure (2) has very adequately summarized the possibilities of small variable dosage sprayers. The machine described here has been used for two seasons using many herbicides including 2,4-D, MCP, 4(MCPB), simazine, and DNBP. It has been found particularly useful for assessing the approximate rate of application at which a given herbicide will kill a particular weed species under field conditions.

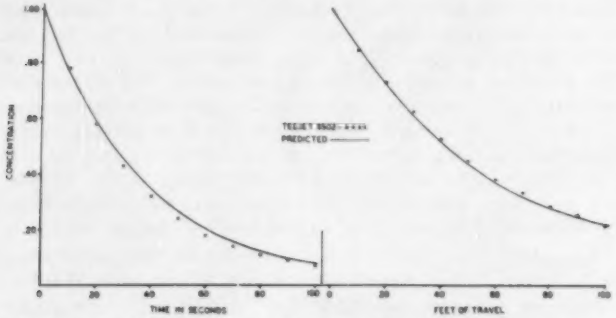

Figure 3. Calibration curves showing the actual and predicted performance of the variable dosage sprayer with time and with distance travelled.

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Loss of Geotropism and Modification of Root Hair Development Produced by Amitrole[1]

B. Counts[2]

Although a number of physiological effects of amitrole (3–amino–1,2,4–triazole) on plants have been reported (1,2) there appears to be nothing in the literature on its effect on germination and seedling development in cotton.

In the experiments reported here, 100 acid-delinted seeds of cotton, *Gossypium hirsutum*, variety Acala 4–42, were soaked in water containing 200 ppm of technical amitrole[3] for two hours. The seeds were distributed to four petri dishes containing filter paper and four ml of tap water. As germination progressed, additional water was added when needed. Controls consisted of the same number of seeds soaked in tap water. All experiments were conducted at room temperature (24–26°C).

The germination percentages after 48, 72 and 96 hours were: treated 52, 84, and 96; untreated, 60, 80, and 96, respectively. However, the emerging radicles lacked the usual curvatures, indicating a lack of geotropic response. Root hairs varied in number from none to many on different seedlings, but all which appeared were short and thick. No normal root hairs were found. The treated seedlings died within two weeks of germination indicating the deformed root hairs were unable to absorb water and nutrients. The experiment was repeated eight times with identical results.

Further evidence of the loss of geotropism was obtained from experiments in which treated seeds were planted in soil with their

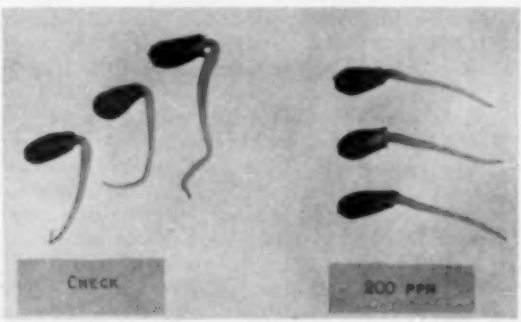

Figure 1. Effect of amitrole on radicles of cotton seedlings. Left, Check; right, soaked 2 hours in solution of 200 ppm amitrole.

[1]Received for publication July 6, 1960.

[2]Assistant Specialist, Botany Department, University of California at Los Angeles. U. S. Cotton Field Station, Shafter, California.

[3]Technical amitrole supplied by American Cyanamid Company.

micropyles pointed either upward, horizontal or downward. Upon germination, the growth direction of the radicle was found to be determined by the direction in which the micropyle had been pointed (Figure 1). Deviation from these directions occurred only when the radicle encountered a physical obstacle.

Cotton seed soaked for two hours in 100 ppm NPA (*N*–1–naphthylphthalamic acid) showed a similar loss of geotropism, but the root hair development was normal. A similar loss of geotropism in bean seedlings following treatment with NPA has been reported (3).

These experiments raise the questions (1) to what extent are these effects of amitrole on cotton seedlings due to interference with normal auxin physiology; and (2) if applied as a defoliant to mature cotton plants, is amitrole translocated to the seeds in sufficient amounts to affect their subsequent development.

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Toxicity of Simazine to Seedling Oat Plants[1]

T. J. Sheets[2]

In a preliminary experiment young oat plants which grew in culture solution containing 0.05 ppmw of 2–chloro–4,6–bis (ethylamino)–*s*–triazine (simazine) for 60 hours were injured but not killed. The objective of the experiment reported here was to study the relations among (a) the concentration of simazine in the culture solution, (b) the concentration of simazine in the oat plant, and (c) the reduction in weight of plants following treatment.

Seeds of oat, variety Kanota, were germinated in autoclaved sand. Seedlings which were 7 to 8 cm high were selected and transferred to 1-pint, wide-mouth mason jars containing 400 ml of nutrient solution 1 of Hoagland and Arnon (2). Four plants were used per jar. The solutions were replaced 5 days after transfer with 400 ml of culture solution containing 0.05 ppmw of C^{14} ring-labeled simazine (0.082 μC). The plants were exposed to the simazine solutions for 0, 36, 60, and 108 hours. One series of plants was harvested at the time the treatments were initiated. The experiment was replicated five times.

At the end of the exposure times, the roots were rinsed for 1 minute in each of three beakers of distilled water; and the plants were transferred to nutrient solution without simazine. Except for the short time during which the roots were washed and the plants transferred to fresh solutions, the cultures were maintained in an environmental chamber under a schedule of 12 hours of light and 12 hours of darkness. The day and night temperatures were 28 ± 2 and 24 ± 2°C respectively. The relative humidity was not controlled. Nine days after the transfer to the simazine solutions, the plants were separated into tops and roots; and the plant parts were dried in the oven at 60°C. The dry plant tissue was weighed and ground in a Wiley mill with a 40-mesh screen. The ground samples were packed uniformly in nickel-plated planchets and counted with a thin-window Geiger-Muller tube and a Tracerlab "1000" scaler. Counts were corrected for self-absorption and converted to specific activities. Sufficient counts were made to maintain precision within about 6 percent error.

Dry weights and specific activities (counts per minute per milligram of dry tissue) of oat tops and roots at the time of harvest 9 days after the initial exposure are recorded in Table 1. The specific activity varied directly with the exposure time. For all exposures the specific activity of tops was greater than that of roots. The dry

[1]Received for publication July 11, 1960. Cooperative investigations of the Crops Research Division, Agricultural Research Service, U.S. Department of Agriculture, and the California Agricultural Experiment Station.

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Table 1. Dry weights and specific activities at harvest 9 days after treatment.

Plant part	Exposure time hours	Dry weight milligrams	Specific activity cpm/milligram
Oat tops	0[a]	123	—
	0[b]	368	—
	36	270	2.3
	60	230	4.5
	108	185	9.2
Oat roots	0[a]	99	—
	0[b]	164	—
	36	124	0.7
	60	110	1.1
	108	87	1.6

[a]Harvested at the time of initial exposure.
[b]Harvested 9 days after initial exposure.

weight of four oat seedlings (one culture) was reduced about 27 per cent by 1.3 micrograms of simazine (C^{14} expressed as simazine) in the tops. Removal by transport upward and by diffusion outward following transfer of the plants from solutions containing simazine to solutions without simazine probably removed the free herbicide molecules from the roots.

The maximum simazine concentration that can be present in plant tissues without reducing growth is not known. Small quantities might stimulate growth (1). The photochemical activity of isolated chloroplasts was reduced 50 per cent by 4.6×10^{-6} molar simazine (4). In this experiment the dry-weight increase (difference between the dry weight at treatment and at harvest) of oat tops was reduced 50 per cent by 7.2 micrograms of simazine (C^{14} expressed as simazine) per gram of tissue (7.2 ppmw) at harvest.

Weights of oat tops and roots were reduced by all exposure treatments (Table 1). However, only a small percentage of the simazine which was in the culture solution at the initiation of the experiment was present in the plants at the time of harvest (Table 2). A portion

Table 2. Counts per minute in oat tops and roots at harvest expressed as a per cent of the counts per minute in the culture solution initially.

Exposure time hours	Tops per cent	Roots per cent	Tops and roots per cent
36	0.64	0.09	0.73
60	1.06	0.12	1.18
108	1.74	0.14	1.88

of the herbicide or C^{14}-labeled metabolites probably diffused out of the roots after transfer to solutions without simazine. Also, C^{14}-labeled carbon dioxide might have been evolved from the plants (3). Therefore, the values in Table 2 do not necessarily represent the amount of simazine absorbed by the plant. However, the amount required in the plant tissues to reduce plant weight was less than 2 per cent of the simazine available for absorption.

Conditions of this experiment were markedly different from those which occur in the field. However, weedy species as well as crop plants probably absorb only a small fraction of herbicides available in the soil solution. In addition, adsorption by colloidal surfaces in the soil and root distribution in relation to the position of the herbicides in the soil profile tremendously decrease the efficiency of soil-applied herbicides.

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News and Notes

The next meeting of the Weed Society of America will be held at the Hotel Jefferson, St. Louis, Missouri, December 11 to 14, 1961. The Society will be the guest of the North Central Weed Control Conference. The officers are: President, K. P. Buchholtz, University of Wisconsin, Madison; Vice-President, Warren C. Shaw, U.S.D.A., Beltsville, Maryland; Secretary, F. W. Slife, University of Illinois, Urbana.

The North Central Weed Control Conference will hold no program meeting in 1961, when it is host to the Weed Society of America at St. Louis. The officers for 1961 are: President, E. K. Alban, Department of Horticulture, The Ohio State University, 1827 Neil Ave., Columbus 10; Vice-President, Sig Bjerken, Minnesota Dept. of Agriculture, 1097 Randolph Ave., St. Paul 5; Secretary-Treasurer, J. D. Furrer, Department of Agronomy, University of Nebraska, Lincoln 3. The 1960 Proceedings, which includes the printed Research Reports, can be obtained from the Secretary-Treasurer for $4.00. The 1962 meeting will be held in St. Paul, Minnesota, December 3, 4, and 5.

The next meeting of the Northeastern Weed Control Conference will be held January 3, 4, and 5, 1962 at the Hotel New Yorker, New York City. The officers for 1961 are: President, Lawrence Southwick, Dow Chemical Company, Midland, Michigan; Vice-President, D. A. Schallock, Extension Weed Control Specialist, Rutgers University, New Brunswick, N. J.; Secretary, P. W. Santelmann, Department of Agronomy, University of Maryland, College Park; Treasurer, J. A. Meade, Department of Agronomy, University of Maryland, College Park. The Proceedings for 1960 can be obtained for $4.50, and the Supplement, which contains the registration list and the Research Committee report, for $2.00, from the Secretary.

The next meeting of the Southern Weed Conference will be held at the Hotel Patton, Chattanooga, Tennessee, January 17, 18, and 19, 1962. The officers for 1961 are: President, Walter K. Porter, Jr., Delta Experiment Station, Stoneville, Mississippi; Vice-President, John T. Holstun, Jr., Crops Research Division, ARS-USDA, Stoneville, Mississippi; Secretary-Treasurer, Robert E. Frans, Department of Agronomy, University of Arkansas, Fayetteville. The Proceedings for 1961 can be obtained from the Secretary-Treasurer for $5.00.

The next meeting of the Western Weed Control Conference will be held March 20, 21, 22, 1962, at the Flamingo Hotel, Las Vegas, Nevada. The officers for 1961 are: President, W. R. Furtick, Oregon State College, Corvallis; Vice-President, Eugene Heikes, Montana State College, Bozeman; Secretary-Treasurer, E. J. Bowles, 3239 Mayfair Boulevard, Fresno, California. The Research Progress Report for 1961 can be obtained from the Secretary for $2.00.

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BIBLIOGRAPHY OF WEED INVESTIGATIONS
for
July, August, September, 1960

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Prepared By
UNITED STATES DEPARTMENT OF AGRICULTURE
Agricultural Research Service
Crops Research Division
Crops Protection Research Branch

This bibliography of current literature on weeds and their control is issued as a service to those engaged in weed research in the United States and other countries. In issuing this bibliography the Crops Research Division, Agricultural Research Service, U.S.D.A. does not assume responsibility for the subject matter cited.

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